

THE BOTANICAL GAZETTE

EDITOR
HENRY CHANDLER COWLES

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WITH NINE PLATES AND FIVE HUNDRED AND FIFTY-TWO FIGURES



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THE BOTANICAL GAZETTE

September 1932

CHROMOSOMAL CONFIGURATIONS OF OENOTHERA SPECIES AND CROSSES AND THEIR PROBABLE SIGNIFICANCE

J. THERON ILLICK

The purpose of this paper is to tabulate all chromosomal configurations of the *Oenothera* species and crosses that have so far been observed and to discuss the significance of chromosomal cohesion.

The tabulations of each form include the name of the investigator, the year reported, and, if available, the source of material and the genetical constitution of each. The writer does not claim that these tables are complete in every instance, but an attempt is made to cover all the work available to date.

Such a series of tables, it is felt, would be of great advantage in any attempt to understand the significance of closed circles so characteristic of the *Oenothera* species. In connection with each table there is given a brief discussion of the forms therein listed. In these discussions there are emphasized in particular two points based upon the data presented in these tables: (1) the variability in chromosomal configurations in any particular *Oenothera* species; and (2) the assumption that closed circles are due to genic action and therefore inherited as are phenotypical characters.

Table I (7 pairs) consists of nineteen different species, each of which, as observed by different investigators, has shown a complete pairing of the fourteen chromosomes. These nineteen *Oenotheras* consist of six species, three mutants, one half-mutant, six segregates, and three hybrid derivatives, some of which have been recognized

TABLE I
CHROMOSOME CONFIGURATION WITH 7 PAIRS

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
<i>lamarckiana</i> *	1924, BOEDIJN	Original strain of DEVRIES	Unstable; isogamous (pollen and egg have same qualities); gives about 1½% "mutants"; of its gene mutations, none has repeated itself except <i>funifolia</i> which appeared in <i>O. pratincola</i> (1919) and in <i>lamarckiana</i> (56); complex <i>velans.gaudens</i> . Also shows ⊙ 12 and 1 pair as most common configuration; or ⊙ 10 and 2 pairs; or 3 pairs and chain; or chain of 14. (There has been expressed some doubt as to correctness of the presence of 7 pairs.)
<i>pervirens</i> *	1929, ILLICK	SHULL	Mutant by SHULL; assumed to be the same genetically as <i>lamarckiana</i> except it has no red pigment and shows a higher percentage of germination (56). Also shows ⊙ 12 and 1 pair as most common condition; or ⊙ 14.
<i>blandina</i>	1925, CLELAND	DEVRIES	Direct mutant of <i>lamarckiana</i> ; first appeared from cross between <i>lata</i> and <i>semilata</i> and is homozygous in crosses; no lethals and is constant except for occasional mutant <i>spiralis</i> (30); homozygous race (DEVRIES); STOMPS (62) says it possesses a greater faculty for mutation than had hitherto been believed; belongs to <i>velutina</i> class.
<i>rubrinervis</i> *	1908, GATES	GATES	HÅKANSSON calls it half-mutant of <i>lamarckiana</i> ; in 1907 gave the mutant <i>rubricalyx</i> ; gives homozygous segregate <i>deserens</i> and in appearance is difficult to distinguish from <i>deserens</i> ; CLELAND calls his forms homozygous strains; GATES claims it is heterozygous for <i>laeta.velutina</i> (25): Also shows ⊙ 6 and 4 pairs as the more common configuration.
<i>deserens</i>	1925, CLELAND 1930, HÅKANSSON	DEVRIES ?	Alethal segregate from half-mutant <i>rubrinervis</i> and difficult to distinguish from <i>rubrinervis</i> ; homozygous race (DEVRIES); belongs to <i>laeta</i> class.
<i>fragilis</i>	1929, HOEPPENER and RENNER	?	A <i>deserens</i> -like homozygous form from all hybrids containing <i>sub-velans</i> .

* Indicates variable configuration of chromosomes.

TABLE I—Continued

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
rubricalyx sulfurea	1929, ILICK	SHULL	Hybrid derivative of <i>rubricalyx</i> -budded <i>brevistylis</i> (or <i>rubricalyx</i> -budded <i>nanella</i>) × <i>franciscana sulfurea</i> .
latifrons	1928, CLELAND	SHULL (DAVIS)	Alethal segregate from half-mutant <i>rubricalyx</i> ; homozygous race (DE VRIES).
hookeri	1924, SCHWEMMLE 1928, CLELAND 1929, HOEPPENER and RENNER	? DEVRIES ?	Very stable; has given <i>franciscana</i> (26) which is also a stable form; wild species; isogamous; complex <i>hookeri.hookeri</i> .
franciscana*	1928, CLELAND	SHULL (DAVIS)	Arose as segregate from <i>hookeri</i> (26); very stable, genetically more so than <i>grandiflora</i> ; has never thrown any mutants and has shown high percentage of pollen and seed fertility; CLELAND (5) stresses it as pure species; GATES (27) claims it must be heterozygous for a number of characters. Also shows ♂ 4 and 5 pairs; or chain of 4 or more.
	1929, KULKARNI	DAVIS (BART-LETT)	
	1929, HOEPPENER and RENNER	?	
	1931, LELIVELD	STOMPS	
franciscana* sulfurea	1928, CLELAND	SHULL (DAVIS)	Hybrid derivative of <i>franciscana</i> × <i>biennis</i> ; similar to <i>franciscana</i> genetically except it has sulfur flowers; non-disjunction high (7). Also shows ♂ 12 and 1 pair as less common configuration.
	1929, ILICK	SHULL (DAVIS)	
franciscana sulfurea (dwarf seg.)	1928, EMERSON	DAVIS	<i>Franciscana sulfurea</i> , selfed, gives many parental forms and about 1/3 dwarf segregates as this; this segregate "is apparently homozygous for all characters."
grandiflora* Ait.	1929, GERHARD	?	"Complex heterozygous," whose complexes, <i>truncans.acuens</i> , are functional both in pollen and egg cells; <i>acuens</i> is viable in homozygous condition, while <i>truncans</i> is not; consequently <i>grandiflora</i> does not breed true but splits off in each generation 1/3 <i>ochracea</i> (<i>acuens.acuens</i>). <i>Grandiflora</i> forms twin hybrids with other species. Also shows ♂ 14; or ♂ 12 and 1 pair.
ochracea	1929, GERHARD	?	See above; complex <i>acuens.acuens</i> .

* Indicates variable configuration of chromosomes.

TABLE I—Continued

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
grandiflora* B	1909, DAVIS 1928, CLELAND 1929, ILLICK	DAVIS SHULL (DAVIS) SHULL (DAVIS)	Very stable wild species; has complex <i>acuens.truncans</i> . Also shows \odot 4 and 5 pairs; or \odot 14.
lutescens	1929, HOEPPENER and RENNER	HERIBERT-NILSSON	Hybrid derivative of <i>suaveolens</i> \times <i>biennis</i> from culture of HERIBERT-NILSSON of <i>lata</i> -like plants in 1912; larger flowers than <i>lamarckiana</i> ; complex <i>flavens.flavens</i> .
flava*	1926, OEHLKERS	OEHLKERS	F ₁ segregate of <i>O. suaveolens</i> \times <i>strigosa</i> ; segregates many new types. Also shows \odot 4 and 5 pairs.
twin hybrid of recidiva	1929, KULKARNI	DAVIS	Twin hybrid from <i>recidiva</i> , selfed; stunted gray non-flowering plant.
purpurata	1929, RUDLOFF	?	Species.

*Indicates variable configuration of chromosomes.

genetically to be either stable or unstable, homozygous or heterozygous.

Eight of the total of nineteen have shown variable chromosomal configurations. Three of these are *rubrinervis*, *franciscana*, and *grandiflora* Ait. GATES (25) calls *rubrinervis* heterozygous for *laeta.velutina* and has observed two types of configurations, 7 complete pairs and 4 pairs and \odot 6, while CLELAND (8) calls his strains homozygous and observes 4 pairs and \odot 6. *Franciscana*, a segregate from *hookeri* and very stable genetically, CLELAND (5) stresses as a pure or homozygous species, while GATES claims his plants must be heterozygous for a number of factors. The chromosomal configurations for this species, seen by four different observers, consist either of 7 pairs, 5 pairs and \odot 4, or a chain of 4 or more. *Grandiflora* Ait., GERHARD (35) calls a "complex heterozygote" and has seen three different configurations, 7 pairs, 1 pair and \odot 12, and \odot 14. The complex present is *truncans.acuens* and is the same as for *grandiflora* B, which shows either 7 pairs, 5 pairs and \odot 4, or \odot 14.

Assuming that these statements are correct, it seems safe to con-

¹ Throughout this paper, this symbol indicates a closed circle of chromosomes.

clude that neither heterozygosity nor homozygosity is concerned in the formation of closed circles.

Fifteen of the total of nineteen species may be divided into three groups from the standpoint of their known genetical relationship. In group 1, we have *lamarckiana*, which has been reported once to have shown 7 complete pairs, the mutants of *lamarckiana*, *pervirens*, and *blandina*, and half-mutant *rubrinervis*; also *deserens* (an aléthal segregate from *rubrinervis*), *fragilis* (a *deserens*-like homozygous form), and *rubricalyx sulfurea* and *latifrons*, both indirectly related to *rubrinervis* through *rubricalyx*. In group 2 there are *hookeri* with 7 complete pairs, *franciscana*, a segregate from *hookeri*, *franciscana sulfurea*, a hybrid derivative of *franciscana* × *biennis*, and *franciscana sulfurea*, a dwarf segregate of *franciscana sulfurea*. In group 3, *grandiflora* Ait. has shown 7 pairs and gives the segregate *ochracea*. *Grandiflora* B is the same as *grandiflora* Ait., at least so far as its reported complex is concerned. The ancestral forms of the other four *Oenotheras*, so far as is known, have not shown complete pairing of their chromosomes.

From the data presented, it appears that any one chromosomal configuration is not constant for any one species but only relatively constant. In no instance, moreover, so far as known, were there observed any phenotypical characters corresponding to these various chromosomal configurations.

It is also to be noted that the members of each group into which fifteen of the nineteen individuals can be arranged, not only have shown 7 pairs of chromosomes but are known to be genetically related. Such a characteristic might well be interpreted as being due to an inherited factor as are the phenotypical characters.

Table II (5 pairs and ⊙ 4) includes a total of six different *Oenotheras* consisting of one species, one mutant, one half-mutant, and three segregates. They all have also shown other configurations. Three of the six have not been mentioned in table I.

Grandiflora is considered a very stable species; *franciscana* is said by GATES (26) to be heterozygous, while by CLELAND (5) it is called a pure species; *rubricalyx*, pure, is spoken of as a perpetual heterozygote possessing one of the zygote lethals of *lamarckiana*; and *flava* is unstable, segregating many new types. It would appear, therefore,

that genetical stability does not prevent the formation of closed circles, nor is it likely that heterozygosity is a factor in their forma-

TABLE II
CHROMOSOME CONFIGURATION WITH 5 PAIRS AND \odot 4

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
<i>grandiflora</i> * B	1919, CLELAND	SHULL	See table I. Also shows 7 pairs; or \odot 14.
<i>franciscana</i> *	1922, CLELAND	DAVIS (BART-LETT)	See table I. Also shows 7 pairs; or chain of 4 or more.
	1928, KULKARNI	DAVIS (BART-LETT)	
	1929, HOEPPENER and RENNER 1931, LELIVELD	? STOMPS	
<i>aurata</i> *	1928, CLELAND 1929, ILLICK	SHULL SHULL	Segregate from <i>franciscana</i> \times <i>biennis</i> , in other words, an F_2 from cross between <i>vetaurea</i> and sulfur (SHULL); breeds true; has <i>lamarckiana</i> in its ancestry. Also shows \odot 12 and 1 pair; or \odot s 10 and 4.
<i>rubricalyx</i> ,* pure	1927, SHEFFIELD 1929, GATES and SHEFFIELD	GATES GATES	Mutant (Mendelian dominant) which occurred in culture of <i>rubrinervis</i> in 1907; has one of the zygote lethals found in <i>lamarckiana</i> and, like mut. <i>rubrinervis</i> and mut. <i>erythrina</i> , is a perpetual heterozygote which segregates in a 2:1 ratio in every generation into heterozygous <i>rubricalyx</i> and homozygous <i>latifrons</i> (from germ cells free of zygote lethals). Complex <i>rubens-tingens</i> . Also shows 4 pairs and \odot 6; or \odot s 6 and 8; or variable.
<i>flava</i> *	1929, OEHLKERS	OEHLKERS	See table I. Also shows 7 pairs.
<i>rubrisepala</i> *	1930, HÅKANSSON	?	Half-mutant from <i>lamarckiana</i> (plants with unbalanced lethal condition). Also shows 4 pairs and \odot 6.

* Indicates variable configuration of chromosomes.

tion. The variations in configurations, moreover, are not known to be related to any observed phenotypical characters.

On the other hand, it is to be noted that those forms that are

known to be genetically related, as *franciscana* and *hookeri* (7 pairs), *franciscana* and *aurata* (5 pairs and \odot 4), *aurata* and *lamarckiana* (1 pair and \odot 12), and *rubricalyx* and *rubrinervis* (4 pairs and \odot 6), have each one known chromosomal configuration in common, which

TABLE III
CHROMOSOME CONFIGURATION WITH 4 PAIRS AND \odot 6

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
rubrinervis*	1908, GATES 1925, CLELAND 1928, CLELAND 1928, HÅKANSSON 1929, ILICK	GATES SHULL SHULL (DAVIS) ? SHULL	See table I. HÅKANSSON studied ovules. Complex <i>paenvelans.subvelans</i> . Also shows 7 pairs as less common configuration.
rubricalyx,* pure	1927, SHEFFIELD 1929, GATES and SHEFFIELD	GATES GATES	See table II. GATES and SHEFFIELD studied megaspore development. Also shows 5 pairs and \odot 4; or \odot s 6 and 8; or variable.
rubrisepala*	1928, HÅKANSSON	?	See table II. Studied ovules. Also shows 5 pairs and \odot 4.
erythrina	1928, CLELAND	DAVIS	Half-mutant of <i>lamarckiana</i> and produces <i>decipiens</i> which is a homozygous race with no lethals and which breeds true and throws no mutants; perpetual heterozygote.
stenophylla* (selfed)	1932, ILICK	SHULL	Mutant by DeVRIES from <i>lamarckiana</i> ; <i>stenophylla</i> \times <i>angustifolia</i> gives <i>lamarckiana</i> ; narrow leaf. Also shows \odot 12 and 1 pair as less common configuration.
"mut. sulfurea"	1928, CLELAND	SHULL	All showed this configuration except one 21-chromosome plant.

* Indicates variable configuration of chromosomes.

permits the assumption that closed circles are the result of genic action.

Table III (4 pairs and \odot 6) includes six *Oenotheras* consisting of three mutants and three half-mutants, four of which show variable chromosomal configurations. One of these four, *stenophylla*, has not been mentioned in previous tables. *Erythrina*, a perpetual heterozygote and half-mutant of *lamarckiana*, and "mut. sulfurea" are the only ones which have thus far shown only single types of chro-

mosomal configurations. With the appearance of these variable chromosomal configurations no observed phenotypical changes are known to have been recorded for the individuals concerned.

TABLE IV
CHROMOSOME CONFIGURATION WITH 3 PAIRS AND \odot 8

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
<i>lamarckiana</i> *	1931, LELIVELD	STOMPS	See table I. Configuration here is 3 pairs and chain; also shows \odot 12 and 1 pair as most common configuration; or \odot 10 and 2 pairs; or chain of 14; or 7 pairs.
<i>rubricalyx</i> Afterglow	1925, CLELAND 1931, EMERSON	SHULL ?	CLELAND (13) believes this "Afterglow" of SHULL's arose through segmental interchange in pure <i>rubricalyx</i> . Complex modified <i>velans</i> . ⁴ <i>latifrons</i> .
<i>germanica</i> *	1931, LELIVELD	STOMPS	Offspring of BOEDIJN's plants. Configuration here consists of 3 pairs and loose chromosomes; also shows \odot 14; or \odot 12 and 1 pair; or \odot 10 and 2 pairs, these three configurations "in one and the same slide" (46).
<i>distans</i>	1930, HÅKANSSON	?	"From $2n+1$ <i>nilens</i> " (18); also called $2n+1$ or 15 chromosome mutant (BOEDIJN) and (GATES).
<i>simplex</i> elongata	1930, HÅKANSSON	?	"A segregate of <i>simplex</i> from $2n+1$ <i>oblonga</i> " (18); " <i>simplex</i> agrees with <i>mut. velutina</i> (<i>blandina</i>) in having very few empty seeds and in not giving twin hybrids. While <i>blandina</i> forms hybrids of <i>velutina</i> type, the hybrids of <i>simplex</i> are of <i>laeta</i> type; also <i>simplex</i> retains the mutability of <i>lamarckiana</i> , which <i>blandina</i> has lost" (30).

* Indicates variable configuration of chromosomes.

It should be noted that *rubrinervis*, with 4 pairs and \odot 6, gives *mut. rubricalyx* also with 4 pairs and \odot 6; and *stenophylla* with 1 pair and \odot 12 is a mutant from *lamarckiana* which also commonly shows a similar configuration. These instances indicate that inherited factors are possibly involved in the production of closed circles as are phenotypical characters.

Table IV (3 pairs and \odot 8) includes five *Oenotheras* consisting of two species and three segregates. Two of the five, it will be noted, have shown variable configurations. One of these two, *germanica*, has not been mentioned in the previous tables. It is included in this table although the complete configuration was not observed. It showed 3 pairs and loose chromosomes. Further study might make it necessary to place it with another group of configurations.

Germanica is interesting in that LELIVELD (46) found three different configurations (\odot 14, \odot 12 and 1 pair, and \odot 10 and 2 pairs) "in one and the same slide." It would therefore be difficult to explain closed circles in *germanica* on the basis of observed genetical behavior. These three configurations of *germanica* in the same slide, LELIVELD claims are a "consequence of fixation." Concerning this point also see page 43 of this paper.

Rubricalyx Afterglow is reported to have come from *rubricalyx* by segmental interchange (CLELAND 13), and *rubricalyx* is a mutant of *rubrinervis* which in turn is a half-mutant of *lamarckiana*, and since *rubricalyx* Afterglow and *lamarckiana* have shown one configuration common to both (3 pairs and \odot 8), it might be supposed that similar heritable factors are involved in each instance.

The two forms which have shown more than one type of configuration have never been known to show any phenotypical variations corresponding to these configurations.

Table V (2 pairs and \odot 10) includes four individuals consisting of three species and one hybrid derivative, all of which have shown variable chromosomal configurations. Two of these were not included in the previous tables.

Lamarckiana, which is unstable genetically and heterozygous, has been said to have shown to date five different chromosomal configurations; *muricata*, which is stable genetically and permanently heterozygous, has shown three different chromosomal configurations; while *germanica* has shown three types of configurations, \odot 14, \odot 12 and 1 pair, and \odot 10 and 2 pairs, "in one and the same slide" (LELIVELD 46), as well as 3 pairs and loose chromosomes.

In no instance where variation in configurations occurred was there observed any phenotypical changes with which they might be associated.

Table VI (1 pair and \odot 12) includes twenty-nine different *Oenotheras* consisting of eleven species, eleven mutants, five hybrid derivatives, and two segregates. Sixteen of these show variable chromosomal configurations, seven of which have not been previously mentioned.

TABLE V
CHROMOSOME CONFIGURATION WITH 2 PAIRS AND \odot 10

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
<i>lamarckiana</i> *	1931, LELIVELD	STOMPS	See table I. Five cases of this configuration seen. Ovules and pollen studied by LELIVELD. Also shows \odot 12 and 1 pair; or chain of 14; or 3 pairs and chain; or 7 pairs.
"heterozygous for 7 characters"*	1928, CLELAND	SHULL	One plant showed this configuration and another showed \odot 12 and 1 pair.
<i>germanica</i> *	1931, LELIVELD	STOMPS	See table IV. Besides this configuration it also shows \odot 14; or \odot 12 and 1 pair, all three seen in same slide. It also shows 3 pairs and loose chromosomes.
<i>muricata</i> *	1931, LELIVELD	STOMPS	Stable genetically; heterogamous (pollen and egg carry different qualities); possibly from <i>biennis</i> or simpler form; has segregated out <i>novae-scotiae</i> ; called permanent heterozygous wild species (10); small flowers and microspores. Complex <i>curvans.rigens</i> . Also shows 1 pair and chain of 12; or \odot 14.

* Indicates variable configuration of chromosomes.

The presence of such variations again emphasizes the fact that chromosomal configurations are not necessarily fixed for any one species. With the appearance of such variations in configurations, moreover, there have been recorded no corresponding phenotypical differences which would permit the conclusion that closed circles are related to any particular phenotypical character.

Eighteen of the twenty-nine of this table can be placed genetically into four groups depending upon common parentage. In each instance the individual members show one or more different configura-

TABLE VI
CHROMOSOME CONFIGURATION WITH 1 PAIR AND \odot 12

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
lamarckiana*	1925, CLELAND	SHULL (DE VRIES)	See table I. <i>Lamarckiana</i> of HERIBERT-NILSSON is white-nerved and by RENNER called r- <i>lamarckiana</i> . HÅKANSSON in 1928 studied ovules. LELIVELD studied both pollen mother cells and ovules. Also shows \odot 10 and 2 pairs; or chain of 14; or 3 pairs and chain; or 7 pairs.
	1926, HÅKANSSON	HERIBERT-NILSSON	
	1928, HÅKANSSON	?	
	1929, CLELAND	RENNER (r- <i>lamarckiana</i>)	
	1929, CLELAND	DEVRIES	
	1929, ILICK	SHULL (DE VRIES)	
	1931, LELIVELD	STOMPS	
mut. from lamarckiana	1932, ILICK	SHULL	Gene mutant by SHULL.
vetaurea sup-plena (selfed)	1932, "	"	"Old gold" and "double flower" from <i>lamarckiana</i> .
angustifolia	1932, "	"	Gene mutant from <i>lamarckiana</i> by DEVRIES; <i>angustifolia</i> \times <i>stenophylla</i> gives <i>lamarckiana</i> ; narrow leaf.
pervirens*	1929, "	"	See table I. The one of 1932 was a selfed individual and showed \odot 14 and \odot 12 and 1 pair in cells four sections apart. Also shows 7 pairs.
	1932, "	"	
stenophylla* (selfed)	1932, "	"	See table III. Also shows \odot 6 and 4 pairs as more common condition.
bullata, pure	1932, "	"	Gene mutant from <i>lamarckiana</i> by SHULL.
acutifolia	1931, BRITTINGHAM	"	Gene mutant from <i>O. lamarckiana</i> cross-bred line maintained for 23 years; mutant gene is from outside first linkage group; characterized by narrower, more sharply pointed rosette leaf and reduction in amount of crinkling; closely resembles parent in floral characters and breeding phenomena. Fifth gene mutation to give typical monohybrid ratios.
lamarckiana cruciata	1929, CLELAND and OEHLKERS	OEHLKERS	Variety of <i>lamarckiana</i> ; has complex <i>velans.gaudens</i> .
lamarckiana sulfurea	1932, ILICK	SHULL	Hybrid derivative from <i>lamarckiana</i> \times <i>biennis</i> .

* Indicates variable configuration of chromosomes.

TABLE VI—Continued

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
fallax	1928, HÅKANSSON	?	Hybrid from <i>lamarckiana</i> × <i>biennis</i> , Chicago (37). Also see table XII for this cross.
suaveolens* (yellow)	1926, OEHLKERS 1928, CLELAND 1929, ILLICK	OEHLKERS RENNER (DE VRIES) SHULL	Resembles <i>biennis</i> in breeding behavior in throwing unlike reciprocal hybrids; wild type. Complex <i>flavens.albicans</i> . Also shows ⊙ 14.
suaveolens (sulfur)	1929, " 1929, CLELAND and OEHLKERS	" OEHLKERS	Mutant from wild yellow-flowered form. Complex <i>flavens.albicans</i> .
albata*	1926, OEHLKERS	OEHLKERS	F ₁ segregate of <i>O. suaveolens</i> × <i>strigosa</i> ; breeds true except in its descendants. Also shows ⊙ 14.
pratincta,* Bartlett	1929, ILLICK	SHULL	Wild species; closely resembles <i>biennis</i> genetically in giving unlike reciprocal hybrids (egg and sperm lethals). Also shows ⊙ 14.
simulans*	1929, KULKARNI	DAVIS	Mutant strain of <i>pratincta</i> (Bartlett). Only two types of gametes produced. Also shows ⊙ 14.
franciscana sulfurea*	1923, 1924, CLELAND	"	See table I. Also shows 7 pairs as more common condition.
aurata*	1928, CLELAND	SHULL	See table II. Also shows ⊙ 4 and 5 pairs; or ⊙s 10 and 4.
gigantea (diploid)	1928, HÅKANSSON	?	Mutant from Swedish <i>lamarckiana</i> by HERIBERT-NILSSON, 1907; non-disjunction frequent (30); HÅKANSSON studied ovules.
"heterozygous for 7 characters"*	1928, CLELAND	SHULL	One plant showed this configuration and another showed ⊙ 10 and 2 pairs.
Oe. (rc. V. o)	1932, ILLICK	"	Will be expected to segregate for "outside in."
strigosa*	1926, OEHLKERS	OEHLKERS	"Most nearly related to <i>hookeri</i> , from which it differs in its small flowers, and in its foliage" (26). When used as male parent and crossed with <i>lamarckiana</i> , it gives twin hybrids, <i>laeta</i> and <i>velutina</i> ; wild species and permanently heterozygous. Complex <i>deprimens.stringens</i> . Also shows ⊙ 14 as more common condition.

* Indicates variable configuration of chromosomes.

TABLE VI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
cockerelli*	1926, OEHLKERS	OEHLKERS	Wild species; permanently heterozygous; when used as male parent and crossed with <i>lamarckiana</i> it gives twin types, <i>laeta.velutina</i> ; small flowers and microspores. Complex <i>elongans.curtians</i> . Also shows \odot 14 as more common condition.
ammophila*	1927, SHEFFIELD 1932, ILICK	GATES (RENNER) SHULL	Strain of unknown origin; very similar to <i>eriantha</i> . "Described by FOCH 1904 from German sand-dunes where it had been introduced from America" (31). Complex of SHEFFIELD's plants <i>rigens.percurvans</i> . Also shows \odot 14 as less common condition in several collections from one plant.
rubristachys	1928, HÅKANSSON	?	Studied the ovules.
germanica*	1931, LELIVELD	STOMPS	See table IV. Also shows \odot 14; or \odot 10 and 2 pairs; or 3 pairs and loose chromosomes.
grandiflora* Ait.	1929, GERHARD	DEVRIES	See table I. Also shows \odot 14; or 7 pairs.
muricata*	1931, LELIVELD	STOMPS	See table V. LELIVELD saw six cases of chain of 12 and 1 pair. Also shows \odot 14; or chain of 10 and 2 pairs.
chicagoensis	1931, CLELAND	From three different countries	Name given by RENNER to the " <i>biennis</i> Chicago" of DEVRIES.

* Indicates variable configuration of chromosomes.

tions which have been observed in the parents concerned. Sufficient data on this point are not available for the other eleven of these twenty-nine forms.

The four groups are as follows: (1) The gene mutants from *lamarckiana* (as mut. from *lamarckiana*, *velutina*, *supplena*, *angustifolia*, *pervirens*, *stenophylla*, *bullata*, and *acutifolia*), a variety of *lamarckiana* (*lamarckiana cruciata*), and the hybrid derivatives of *lamarckiana* \times *biennis* (as *lamarckiana sulfurea* and *fallax*), all have shown one or more configurations like *lamarckiana*. Two of these

have been mentioned previously in tables I and III respectively. (2) *Suaveolens* (sulfur) is a mutant from *suaveolens* (yellow), both of which have one configuration in common (1 pair and \odot 12). *Albata*, an F_1 segregate of *O. suaveolens* \times *strigosa*, shows two types of configurations both of which resemble the types shown by its one parent, *suaveolens* (\odot 14 and \odot 12 and 1 pair). (3) *Simulans*, a mutant from *pratincola* (Bartlett), also shows two types of configurations both of which resemble those of the parent, *pratincola* (\odot 14 and \odot 12 and 1 pair). (4) *Franciscana sulfurea*, a hybrid derivative of *lamarckiana* \times *biennis*, shows one configuration, 7 pairs, like one of its parents, *franciscana*; and *aurata* resembles one of its parents, also *franciscana*, in showing 5 pairs and \odot 4.

In a letter from Professor G. H. SHULL under date of June 6, 1929, he says, "in the breeding behavior of this plant (27216 (17) *lamarckiana sulfurea*) the results are the same as if it were an *erythrina* instead of a *lamarckiana*." *Erythrina* has shown a configuration of \odot 6 and 4 pairs and *lamarckiana sulfurea* a \odot 12 and 1 pair. In this instance the breeding behavior of the individual has no apparent relation to the chromosomal configuration. In other words, the fact that any two plants exhibit the same breeding behavior does not necessarily permit the conclusion that their chromosomal configurations are identical. Resemblances in chromosomal configurations between offspring and parents as previously shown, on the other hand, permit the assumption that closed circles are inherited as are ordinary phenotypical characters.

Table VII (\odot s 6 and 8) includes five individuals consisting of one species, three mutants, and one segregate. None of these has been previously mentioned. Four of the five show variable chromosomal configurations.

If *muricata* arose from *biennis*, as has been suggested, then four of the individuals in this table are genetically of the same strain, that is, *biennis*. *Biennis sulfurea* and *biennis cruciata* are both mutants of *biennis* and show at least one of the chromosomal configurations of the parent. The former shows both configurations of *biennis*, as does *novae-scotiae* which is a segregate of *muricata*. The origin of *rubricalyx* from *rubrinervis*, and their possession of a common configuration, have been mentioned in table II.

Such similarity in chromosomal configuration between parent and offspring should not be considered a mere coincidence. On the other hand, it indicates a possible hereditary mechanism for the formation

TABLE VII
CHROMOSOME CONFIGURATION WITH \odot s 6 AND 8

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
biennis L.*	1923, CLELAND	DAVIS (DE VRIES)	Wild species; said by BOEDIJN to be derived from <i>lamarckiana</i> ; gives unlike reciprocal hybrids (egg and sperm lethals); intermediate flower size; permanently heterozygous (CLELAND); STOMPS does not consider it a hybrid and thinks it has been for centuries a constant, pure, and truly uniform species. Complex <i>rubens.albicans</i> . Also shows \odot 14; or \odot 4 and loose chromosomes; or 3 pairs in ovules.
	1924, EMERSON	DAVIS (?)	
	1926, CLELAND	DAVIS (DE VRIES)	
	1928, CLELAND	RENNER (R- <i>biennis</i>)	
	1931, LELIVELD 1931, DARLINGTON	STOMPS Seed growing wild at Camberley	
biennis sulfurea*	1923, CLELAND	DAVIS (DE VRIES)	Mutant of <i>biennis</i> ; also shows \odot 14.
	1925, EMERSON	DAVIS (DE VRIES)	
	1926, CLELAND	DAVIS (DE VRIES)	
biennis cruciata	1932, ILLICK	SHULL	Mutant from <i>biennis</i> by STOMPS; differs from wild <i>biennis</i> in having narrow petals.
novae-scotiae*	1927, SHEFFIELD	GATES	Segregate from <i>muricata</i> which latter has bred true for many years; (<i>muricata</i> possibly from <i>biennis</i>); some non-disjunction; small flowers. Also shows \odot 14 as more common configuration.
rubricalyx,* pure	1929, RUDLOFF	?	See table II. Also shows 4 pairs and \odot 6; or 5 pairs and \odot 4.

* Indicates variable configuration of chromosomes.

of closed circles and permits the assumption of specific genes as causal factors.

Table VIII (\odot s 4 and 10) consists of only one form, *aurata*, a segregate from *franciscana* \times *biennis*, with *lamarckiana* in its ancestry.

Table IX (\odot 14) includes thirty individuals which consist of twenty species, seven mutants, one hybrid derivative, and two seg-

regates. Thirteen of the thirty were not mentioned in the preceding tables and have shown only one type of configuration. The other seventeen have shown various types of chromosomal configurations other than the \odot 14, and have been previously mentioned in at least one of the other eight tables.

Information is available for fifteen of these thirty forms which shows that the configurations of known offspring are identical to those of the parents concerned. *Pervirens*, a mutant from *lamarckiana*, is known to have shown three of the configurations seen in *lamarckiana*. *Biennis* has thrown *biennis sulfurea* as a mutant and possibly *muricata*. The latter in turn has segregated *novae-scoliae*. These all have one configuration in common and in some cases two.

TABLE VIII
CHROMOSOME CONFIGURATION WITH \odot s 4 AND 10

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
aurata*	1927, CLELAND	SHULL	See table II. Also shows 5 pairs and \odot 4; or 1 pair and \odot 12.

* Indicates variable configuration of chromosomes.

Suaveolens (\odot 14) and *strigosa* (\odot 14), two different species, when crossed, give *albata*, which also shows the same configuration. *Pra-tincola* has six mutants in this table, all of which show the same configuration, \odot 14.

It is to be noticed that in the case of *biennis* L. some investigators emphasize it as being permanently heterozygous, while by others it is said to be "a constant, pure, and uniform species."

While some of these forms showing \odot 14 are known to be stable genetically, as *muricata* and *grandiflora*, they are also known to have shown various types of chromosomal configurations. They both have shown three types of configurations, \odot 14, chain of 12 and 1 pair, chain of 10 and 2 pairs and \odot 14, \odot 5 and 4 pairs, 7 pairs, respectively.

There is also *grandiflora* Ait., a "complex heterozygote," with three types of configurations, and showing segregation in each generation of two forms (the third being non-viable), apparently without regard to the type of configuration possessed.

TABLE IX
CHROMOSOME CONFIGURATION WITH \odot 14

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
<i>lamarckiana</i> *	1931, LELIVELD	STOMPS	See table I. Also shows \odot 12 and 1 pair; or \odot 10 and 2 pairs; or 3 pairs and chain; or 7 pairs. Configuration here is chain of 14.
<i>pervirens</i> *	1929, ILICK 1932, ILICK	SHULL SHULL	See table I. Also shows \odot 12 and 1 pair as more common condition; or 7 pairs. Plant of 1932 was a selfed individual and showed \odot 14, and \odot 12 and 1 pair in cells four sections apart.
<i>biennis</i> L.*	1923, CLELAND 1931, DARLINGTON	DAVIS (DE VRIES) From seeds growing wild at Camberley	See table VII. Also shows \odot s 6 and 8; or \odot 4 and loose chromosomes; or 3 pairs and loose chromosomes in ovules.
<i>biennis</i> sulfurea*	1923, CLELAND	DAVIS (DE VRIES)	See table VII. Also shows \odot s 6 and 8.
<i>suaveolens</i> * (yellow)	1926, OEHLKERS	OEHLKERS	See table VI. Also shows \odot 12 and 1 pair.
<i>albata</i> *	1929, OEHLKERS	OEHLKERS	See table VI. Also shows \odot 12 and 1 pair.
<i>pratincta</i> *, Bartlett	1929, KULKARNI	DAVIS	See table VI. Also shows \odot 12 and 1 pair.
<i>simulans</i> *	1929, "	"	See table VI. Also shows \odot 12 and 1 pair.
<i>recidiva</i>	1929, "	"	Mutant of <i>pratincta</i> (Bartlett); carried as selfed line since 1915 and gives two types in every generation, itself (\odot 14) and stunted non-flowering plant (7 pairs).
<i>formosa</i>	1929, "	"	Mutant of <i>pratincta</i> (Bartlett).
<i>pratincta</i> C.	1929, "	"	Strain of <i>pratincta</i> (Bartlett).
<i>pratincta</i> E.	1929, "	"	Strain of <i>pratincta</i> (Bartlett).
<i>pratincta</i> M.	1929, "	"	From <i>formosa</i> \times strain C.

* Indicates variable configuration of chromosomes.

TABLE IX—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
muricata*	1923, CLELAND	DAVIS (DE VRIES)	See table V. Also shows 1 pair and chain of 12; or 2 pairs and chain of 10.
	1926, CLELAND	DAVIS (DE VRIES)	
	1931, LELIVELD	STOMPS	
novae-scotiae*	1927, SHEFFIELD	GATES	See table VII. Also shows \odot s 6 and 8 as less common condition.
cockerelli*	1926, OEHLKERS	OEHLKERS	See table VI. Also shows \odot 12 and 1 pair as less common condition.
	1929, CLELAND and OEHLKERS	OEHLKERS	
strigosa*	1926, OEHLKERS	OEHLKERS	See table VI. Also shows \odot 12 and 1 pair as less common condition.
ammophila*	1927, SHEFFIELD	GATES (RENNER)	See table VI. Also shows \odot 12 and 1 pair as less common condition.
agari*	1927, "	GATES	Small flowers; sterile pollen and non-viable seeds relatively low; very uniform in appearance and behavior except for occurrence of one narrow leaved mutant; no cases of non-disjunction found; will cross with species of the <i>lamarckiana</i> group, while within this group the species can be readily intercrossed, although in nature many of them are self-pollinated (28). Also shows variable chromosome conditions including a \odot 3.
eriensis	1927, "	"	Wild species; small flowers; sometimes non-disjunction; plants remarkably uniform in appearance; collections from six different plants along Lake Erie; so similar to <i>ammophila</i> that it might be considered a subspecies.
angustissima	1929, GATES and SHEFFIELD	GATES (SHARP)	Wild species; small flowers; non-disjunction and double non-disjunction; considered a permanent hybrid as other small flowered species (28).
sinuata L.	1927, SINOTO	?	"A species belonging to the <i>Euoenothera</i> group" (46).
pyncocarpa	1930, CATCHESIDE	GATES	Wild species; small flowers; permanent hybrid; closely related to <i>mutans</i> .

* Indicates variable configuration of chromosomes.

TABLE IX—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
nutans	1930, CATCHESIDE	GATES	Wild species; small flowers; permanent hybrid.
germanica*	1931, LELIVELD	STOMPS	See table IV. Also shows \odot 12 and 1 pair; or \odot 10 and 2 pairs; or 3 pairs and loose chromosomes.
grandiflora B*	1929, CLELAND and OEHLKERS	DEVRIES	See table I. Also shows 7 pairs; or \odot 4 and 5 pairs.
grandiflora* Ait.	1929, GERHARD	DEVRIES	See table I. Also shows \odot 12 and 1 pair; or 7 pairs.
pachycarpa	1930, RUDLOFF	?	Species with complex <i>augens.sub-curvans</i> .
rosea	1924, SCHWEMMLE	?	Subgenus <i>hartmannia</i> .
berteriana	1931, DARLINGTON	RENNER	Species.

* Indicates variable configuration of chromosomes.

The evidence here presented, some of which was mentioned in previous tables, certainly indicates that neither homozygosity nor heterozygosity has any direct bearing on the formation of closed circles. When a careful study is made of the parents and their offspring mentioned in this table they invariably have been shown to possess at least one and possibly several chromosomal configurations in common, which permits the assumption that closed circles are characters, as are the usual phenotypical characters, and as such, the expression of factor activity.

Table X consists of five trisomic individuals none of which was mentioned in the preceding tables. Two, *oblonga* and *pulla*, have shown more than one type of configuration. *Oblonga* is a trisomic mutant from *lamarckiana* or from *scintillans* selfed, and *pulla* is also a trisomic mutant from *lamarckiana*. Of the other three, *lata* and *stricta* (the former a trisomic mutant from *lamarckiana*) have shown the same type of configuration, \odot 13 and 1 pair, while *scintillans* shows 7 complete pairs and one unpaired chromosome.

Table XI includes only those individuals which have shown vari-

able chromosomal configurations, of which there are at present twenty-nine. Since these have already been discussed in one or more

TABLE X

TRISOMICS

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	GENETICAL CONSTITUTION, ETC.
<i>lata</i>	1928, HÅKANSSON	?	Trisomic mutant of <i>lamarckiana</i> ; first discovered by DEVRIES in 1887; almost completely male sterile; forms chromosome fragments in meiotic division of pollen mother cells. "Wherever extra chromosome is present, splitting will occur in offspring no matter with what other features the <i>lata</i> characters may be associated" (26). HÅKANSSON studied ovules. Configuration is \odot 13 and 1 pair.
<i>stricta</i>	1928, HÅKANSSON	?	Shows \odot 13 and 1 pair.
<i>scintillans</i>	1918, HANCE	DEVRIES	Has appeared only rarely; derived from <i>lata</i> and <i>lamarckiana</i> . When selfed "it regularly produces <i>lamarckiana</i> , <i>oblonga</i> and <i>scintillans</i> in varying proportions together with occasional mutants such as <i>lata</i> and <i>nanella</i> " (GATES). Shows 7 pairs and 1 unpaired.
<i>oblonga</i> *	1923, CLELAND	DAVIS	Trisomic mutant from <i>lamarckiana</i> by DEVRIES, may also arise from <i>scintillans</i> selfed. Shows \odot 5 and 5 pairs; or \odot 3; or chains of 5, 7, 9 and others paired.
<i>pulla</i> *	1928, HÅKANSSON	?	Trisomic mutant from <i>lamarckiana</i> ; in heterotypic anaphase the distribution in trisomics is 7 and 8 (HÅKANSSON). Shows mostly \odot 6, 3 pairs and 1 trivalent; other conditions seen but not definitely stated; ovules studied.

* Indicates variable configuration of chromosomes.

of the preceding tables, nothing more need be said at this time except to emphasize (1) the difficulty of assuming that chromosomal configurations are constant for any one species; and (2) that whenever such various configurations have occurred, there were observed no corresponding phenotypical changes which might warrant the con-

TABLE XI
VARIABLE CHROMOSOME CONFIGURATIONS

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
lamarckiana (velans. gaudens)	1924, BOEDIJN	Original strain of DEVRIES	7 pairs (?)	cf tables I, IV, V, VI, IX.
	1925, CLELAND	SHULL (DEVRIES)	⊙ 12 and 1 pair	
	1926, HÅKANSSON	?	⊙ 12 and 1 pair	
	1928, HÅKANSSON	HERIBERT-NILSSON	⊙ 12 and 1 pair	
	1929, CLELAND	RENNER (lamarckiana)	⊙ 12 and 1 pair	
	1929, CLELAND	DEVRIES	⊙ 12 and 1 pair	
	1929, ILICK	SHULL (DEVRIES)	⊙ 12 and 1 pair	
	1931, LELIVELD	STOMPS	⊙ 12 and 1 pair; ⊙ 10 and 2 pairs; chain and 3 pairs; chain of 14; and all or almost all loose	
pervirens mut. (velans. gaudens)?	1929, ILICK	SHULL	⊙ 12 and 1 pair; in another pedigreed plant 7 pairs; in selfed plant ⊙ 14, and ⊙ 12 and 1 pair were seen in same bud four sections apart	cf tables I, VI, IX. ⊙ 12 and 1 pair most common.
stenophylla (selfed)	1932, ILICK	SHULL	⊙ 12 and 1 pair; ⊙ 6 and 4 pairs, the latter seen in two plants of same pedigree	cf tables III, VI. ⊙ 6 and 4 pairs more common.
oblonga	1923, CLELAND	DAVIS	⊙ 5 and 5 pairs; ⊙ 3; chains of 5, 7, 9, and others paired	cf table X.
pulla	1928, HÅKANSSON	?	Mostly ⊙ 6, 3 pairs and 1 trivalent	cf table X. Also other conditions but not definitely stated.
cockerelli (elongans. curtans)	1926, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX. ⊙ 14 more common.

TABLE XI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
<i>biennis</i> L. (rubens. albicans)	1923, CLELAND	DAVIS (DEVRIES)	⊙ 14; ⊙s 6 and 8	cf tables VII, IX. ⊙s 6, 8 more common.
	1925, EMERSON	DAVIS (DEVRIES)	⊙s 6 and 8	
	1926, CLELAND	DAVIS (DEVRIES)	⊙s 6 and 8	
	1928, CLELAND	RENNER (<i>R-biennis</i>)	⊙s 6 and 8	
	1931, LELIVELD	STOMPS	⊙6 s and 8; ⊙ 4 and loose chromosomes; in ovules 3 pairs and loose chromosomes	
	1931, DARLINGTON	Seed growing wild at Camberley	⊙ 14 in plant A and C; ⊙s 6 and 8 plant B	
<i>biennis</i> sulfurea	1923, CLELAND	DAVIS (DEVRIES)	⊙ 14; ⊙s 6 and 8	cf tables VII, IX. ⊙s 6 and 8 more common.
	1925, EMERSON	DAVIS (DEVRIES)	⊙s 6 and 8	
	1926, CLELAND	DAVIS (DEVRIES)	⊙s 6 and 8	
<i>biennis</i> gigas	1931, LELIVELD	STOMPS	Several pairs and loose chromosomes; or chromosomes linked and no pairs; or all chromosomes detached; nothing more definite given	From <i>bien-nis</i> × <i>bien-nis cruci-ata</i> by STOMPS, 1919.
	1929, HOEPFENER and RENNER	RENNER	Several pairs	
<i>suaveolens</i> (yellow) (flavens. albicans)	1926, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
	1928, CLELAND	RENNER (DEVRIES)	⊙ 12 and 1 pair	
	1929, ILLICK	SHULL	⊙ 12 and 1 pair	
<i>strigosa</i> (deprimens. stringens)	1926, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
<i>albata</i>	1926, 1929, OEHLKERS	OEHLKERS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
<i>flava</i>	1926, 1929, OEHLKERS	OEHLKERS	7 pairs; ⊙ 4 and 5 pairs	cf tables I, II. F ₁ seg. of <i>suaveolens</i> × <i>strigosa</i> as is also <i>albata</i> above.

TABLE XI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
pratincola, Bartlett	1920, KULKARNI 1929, ILICK	DAVIS SHULL	⊙ 14 ⊙ 12 and 1 pair	cf tables VI, IX.
simulans	1929, KULKARNI	DAVIS	⊙ 14; ⊙ 12 and 1 pair	cf tables VI, IX.
muricata (curvans. rigens)	1923, CLELAND 1926, CLELAND 1931, LELIVELD	DAVIS (DEVRIES) DAVIS (DEVRIES) STOMPS	⊙ 14 ⊙ 14 ⊙ 14; chain 12 and 1 pair; chain 10 and 2 pairs; and loose or almost loose chromosomes	cf tables V, VI, IX.
novae-scotiae	1927, SHEP-FIELD	GATES	⊙ 14; ⊙s 6 and 8	cf tables VII, IX. ⊙ 14 more common.
agari	1927, SHEP-FIELD	GATES	⊙ 14; or variety of conditions including ⊙ 3	cf table IX. ⊙ 14 very common.
ammophila (rigens. per-curvans)	1927, SHEP-FIELD 1932, ILICK	GATES (RENNER) SHULL	⊙ 14; ⊙ 12 and 1 pair ⊙ 12 and 1 pair	cf tables VI, IX.
rubrinervis (lacta. velutina)	1908, GATES 1925, CLELAND 1928, CLELAND 1928, HÅKANSSON 1929, ILICK	GATES SHULL SHULL (DAVIS) ? SHULL	⊙ 6 and 4 pairs; 7 pairs ⊙ 6 and 4 pairs (one plant) ⊙ 6 and 4 pairs ⊙ 6 and 4 pairs in ovules ⊙ 6 and 4 pairs	cf tables I, III.
rubricalyx, pure (rubens. tingenens)	1927, SHEP-FIELD 1929, GATES and SHEFFIELD 1929, RUDLOFF	GATES GATES ?	⊙ 4 and 5 pairs; ⊙ 6 and 4 pairs ⊙ 4 and 5 pairs; ⊙ 6 and 4 pairs ⊙ 6 and 8	cf tables II, III, VII. Variables also seen.
"heterozygous for 7 characters"	1928, CLELAND	SHULL	⊙ 12 and 1 pair; ⊙ 10 and 2 pairs	cf tables V, VI.
rubrisepala	1928, 1930, HÅKANSSON	?	⊙ 6 and 4 pairs ⊙ 4 and 5 pairs	cf tables II, III.

TABLE XI—*Continued*

NAME OF FORM	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CONFIGURATIONS	REMARKS
germanica	1931, LELIVELD	STOMPS	⊙ 14; ⊙ 12 and 1 pair; ⊙ 10 and 2 pairs (these seen in one and same slide); 3 pairs and loose chains; or all de- tached	cf tables IV, V, VI, IX.
grandiflora B (truncans. acuens)	1909, DAVIS 1919, CLELAND 1928, CLELAND 1929, ILLICK 1929, CLELAND and OEHL- KERS	DAVIS SHULL SHULL (DAVIS) SHULL (DAVIS) DEVRIES	7 pairs ⊙ 4 and 5 pairs 7 pairs 7 pairs ⊙ 14	cf tables I, II, IX.
grandiflora Ait. (trun- cans.acu- ens)	1929, GERHARD	?	⊙ 14; ⊙ 12 and 1 pair; 7 pairs	cf tables I, VI, IX.
franciscana	1922, CLELAND 1928, CLELAND 1928, KULKAR- NI 1929, HOEP- PENER and RENNER 1931, LELIVELD	DAVIS (BART- LETT) SHULL (DAVIS) DAVIS (BART- LETT) ? STOMPS	⊙ 4 and 5 pairs 7 pairs ⊙ 4 and 5 pairs; 7 pairs 7 pairs 7 pairs; chain of 4 or more	cf tables I, II.
franciscana sulfurea	1923, 1924, CLELAND 1928, CLELAND 1929, ILLICK	DAVIS SHULL (DAVIS) SHULL (DAVIS)	⊙ 12 and 1 pair 7 pairs 7 pairs	cf tables I, VI.
aurata	1927, CLELAND 1928, CLELAND 1932, ILLICK	SHULL SHULL SHULL	⊙ 4 and 10 ⊙ 4 and 5 pairs; ⊙ 12 and 1 pair ⊙ 4 and 5 pairs	cf tables II, VI, VIII.

clusion that changes in the formation of closed circles are in any way associated with the appearance of the plant. The two forms, *per-*

virens and *germanica*, each of which has shown in one slide two or more types of configurations, would be especially difficult to explain on this hypothesis.

The members of this table of variables consist of eleven species, nine mutants and half-mutants, five segregates, one hybrid derivative, and three of unknown make-up. It appears, therefore, that the condition governing variable configurations is not restricted to any one genetical type of *Oenothera*.

In view of the data presented thus far, it would be more consistent to assume that closed circles are characters as are the generally recognized phenotypical characters of the *Oenotheras* and as such, the expression of heritable factors.

Table XII consists of F_1 and F_2 individuals of 105 *Oenothera* crosses. Some of these crosses are duplicates in that the parents are the same in name, but the genetical complex in the parents has been recorded as being different.

By carefully checking the chromosomal configurations of the hybrids with those of their parents, it will be found that sixty-four show common configurations in this respect while forty-one have so far shown no resemblance in configurations between the two parents, or the one known parent, and the offspring. Two of these forty-one were trisomics and two had only one parent whose configuration was known. Further study of the thirty-nine diploid individuals may later reveal in some instances configurations which resemble those of one or both parents. Such a condition is not necessary, however, since in certain cases it might be expected that the chromosomal configuration of the offspring would not conform to those of the parents owing to the failure to inherit the proper genic combination.

In one instance the F_1 of (*biennis* \times *rubricalyx*) \times *ammophila*, a triple heterozygote, showed only 7 complete pairs which GATES and SHEFFIELD (34) claim "makes it clear that complete pairing cannot be regarded as a sign of the homozygous condition." Twelve other triple hybrids in this table have also shown 7 complete pairs of chromosomes. Hybridity, as such, apparently acts as no deterrent to complete pairing at diakinesis.

TABLE XII
CHROMOSOME CONFIGURATIONS OF OENOTHERA CROSSES

F ₁ HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
seg. decipiens × grandiflora B	1929, ILLICK	SHULL	⊙ 6 and 4 pairs	♀ stable; no lethals. ♂ 7 pairs; ⊙ 4 and 5 pairs; ⊙ 14. cf tables I, II, IX, XI.
(seg. decipiens × grandiflora B) F ₂	1929, ILLICK	SHULL	7 pairs com- mon; ⊙ 6 and 4 pairs	♀ ♂ cf above. Hybrid showing 7 pairs.
(grandiflora B × seg. decipiens) F ₂	1929, ILLICK	SHULL	7 pairs com- mon; ⊙ 6 and 4 pairs	♀ ♂ cf above. Hybrid showing 7 pairs
grandiflora B × hookeri	1929, CLELAND and OEHLKERS	OEHLKERS	⊙ 14	♀ cf above. ♂ 7 pairs. cf table I. Complex <i>truncans</i> . <i>hookeri</i> ; no factor ex- change except flower size.
grandiflora B × hookeri, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	2 ⊙s 4 and 3 pairs	♀ ♂ cf above. Complex <i>acuens</i> . <i>hookeri</i> and recip. At least four independent fac- tor exchanges between complexes.
grandiflora Ait. × hookeri	1929, GERHARD	?	⊙ 8 and 3 pairs	♀ 7 pairs; ⊙ 14; ⊙ 12 and 1 pair. cf tables I, VI, IX, XI. ♂ cf above.
grandiflora B × lamarckiana, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	⊙ 14	♀ cf above. ♂ 7 pairs (?); 3 pairs and chain; 2 pairs and ⊙ 10; 1 pair and ⊙ 12; chain of 14. cf tables I, IV, V, VI, IX, XI. Com- plex <i>acuens.gaudens</i> ; no factor exchange ex- cept flower size.
grandiflora B × lamarckiana, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	⊙s 10 and 4	♀ ♂ cf above. Complex <i>truncans.re-</i> <i>lans</i> and recip. and <i>truncans.gaudens</i> and recip. No factor ex- change except flower size.

TABLE XII—*Continued*

F ₁ HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
grandiflora B × lamarckiana, and recip.	1929, CLELAND and OEHLKERS	OEHLKERS	⊙s 6 and 4 and 2 pairs	♀ ♂ cf above. Complex <i>acuens.ve-</i> <i>lans</i> ; considerable fac- tor exchange.
grandiflora Ait. × lamarckiana	1929, GERHARD	?	⊙s 6 and 4 and 2 pairs	♀ ♂ cf above. Complex <i>acuens.ve-</i> <i>lans</i> .
lamarckiana × grandiflora Ait.	1929, “	?	7 pairs	♀ ♂ cf above. Complex <i>truncans.ve-</i> <i>lans</i> .
lamarckiana × grandiflora Ait.	1929, “	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex <i>acuens.gau-</i> <i>dens</i> .
(lamarckiana × grandiflora Ait.) F ₂	1929, “	?	⊙ 4 and 5 pairs	♀ ♂ cf above. Complex <i>acuens.ve-</i> <i>lans</i> .
grandiflora B × franciscana	1928, CLELAND	SHULL	⊙ 4 and 5 pairs	♀ cf above. ♂ 7 pairs; 5 pairs and ⊙ 4; chain of 4 or more. cf tables I, II, XI.
grandiflora B × franciscana	1928, EMERSON	?	Configura- tion re- sembles one or other par- ent	♀ ♂ cf above.
franciscana × grandiflora B	1928, CLELAND	SHULL	5 pairs and ⊙ 4	♀ ♂ cf above.
franciscana × grandiflora B	1928, EMERSON	?	Resembles one or other parent	♀ ♂ cf above.
“mut. sulfu- rea” × grandiflora B	1928, CLELAND	SHULL	⊙ 6 and 4 pairs; 7 pairs	♀ ⊙ 6 and 4 pairs. cf table III. ♂ cf above. Hybrid with 7 pairs.
grandiflora B × “mut. sulfurea”	1928, CLELAND	SHULL	⊙ 6 and 4 pairs; 7 pairs	♀ ♂ cf above. Hybrid with 7 pairs.

TABLE XII—Continued

F ₁ HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
grandiflora Ait. × sua- veolens	1929, GERHARD	?	⊙ 4 and 5 pairs	♀ cf above. ♂ ⊙ 14; ⊙ 12 and 1 cf tables VI, IX, XI. Complex <i>flavens.acu-</i> <i>ens.</i>
grandiflora Ait. × sua- veolens	1929, GERHARD	?	⊙ 14	♀ ♂ cf above. Complex <i>flavens.trun-</i> <i>cans.</i>
grandiflora Ait. × muri- cata	1929, GERHARD	?	⊙ 10 and 2 pairs	♀ cf above. ♂ ⊙ 14; chain 12 and 1 pair; chain 10 and 2 pairs; loose or almost loose chromosomes. cf tables V, VI, IX, XI. Complex <i>curvans.trun-</i> <i>cans.</i>
muricata × grandiflora Ait.	1929, GERHARD	?	⊙ s 8, 4 and 1 pair	♀ ♂ cf above. Complex <i>rigens.acuens.</i>
muricata × grandiflora Ait.	1929, GERHARD	?	⊙ 14	♀ ♂ cf above. Complex <i>rigens.trun-</i> <i>cans.</i>
grandiflora Ait. × bien- nis cruciata	1929, GERHARD	?	⊙ s 10, 4	♀ cf above. ♂ ⊙ s 6, 8. cf table VII. Complex <i>rubens.acu-</i> <i>ens.</i>
grandiflora Ait. × bien- nis cruciata	1929, GERHARD	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex <i>albicans.acu-</i> <i>ens.</i>
grandiflora Ait. × cruci- ata	1929, GERHARD	?	⊙ s 10, 4	♀ cf above. ♂ ? Complex <i>truncans.flec-</i> <i>tens.</i>
cockerelli × grandiflora Ait.	1929, GERHARD	?	⊙ 14	♀ ⊙ 14; ⊙ 12 and 1 pair. cf tables VI, IX, XI. ♂ cf above. Complex <i>curtans.trun-</i> <i>cans.</i>
grandiflora Ait. × bien- nis cruciata	1929, GERHARD	?	⊙ 12 and 1 pair	♀ ♂ cf above. Complex <i>albicans.trun-</i> <i>cans.</i>
grandiflora Ait. × bien- nis cruciata	1929, GERHARD	?	⊙ 14	♀ ♂ cf above. Complex <i>rubens.trun-</i> <i>cans.</i>

TABLE XII—*Continued*

F. HYBRIDS UNLESS OTHER- WISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
<i>franciscana</i> <i>sulfurea</i> × <i>latifrons</i>	1928, CLELAND	SHULL	⊙ 6 and 4 pairs	♀ 7 pairs common; 1 pair and ⊙ 12. cf tables I, VI, XI. ♂ 7 pairs. cf table I.
<i>aurata</i> × <i>lati-</i> <i>frons</i>	1928, CLELAND	SHULL	⊙ 4 and 5 pairs; ⊙ 6 and 4 pairs	♀ ⊙ 4 and 5 pairs; ⊙ 12 and 1 pair; ⊙s 10, 4. cf tables II, VI, VIII, XI. ♂ cf above.
<i>aurata</i> × <i>bul-</i> <i>lata</i>	1932, ILLICK	SHULL	⊙ 6 and 4 pairs	♀ cf above. ♂ ⊙ 12 and 1 pair. cf table VI.
<i>rubricalyx</i> × <i>grandiflora</i> B	1925, CLELAND	?	⊙ 8 and 3 pairs	♀ ⊙ 6 and 4 pairs; ⊙ 4 and 5 pairs. cf tables II, III, XI. ♂ cf above.
<i>rubricalyx</i> × <i>hewettii</i>	1923, GATES	GATES	⊙ 5 and 5 pairs	♀ cf above. ♂ Chromosomes may be like <i>franciscana</i> ; did not breed true in GATES's cultures. Both parents distinct species. Mu- tant of this cross un- named.
<i>hewettii</i> × <i>ru-</i> <i>bricalyx</i>	1923, GATES	GATES	⊙ 5 and 5 pairs	♀ ♂ cf above.
<i>ammophila</i> × (<i>biennis</i> × <i>rubrica-</i> <i>lyx</i>) F ₂	1929, GATES and SHEP- FIELD	GATES	⊙ 8 and 3 pairs; ⊙ 4 and 5 pairs or ⊙ 12 and 1 pair	♀ ⊙ 12 and 1 pair; ⊙ 14. cf tables VI, IX, XI. ♂ <i>Biennis</i> shows ⊙s 6, 8; or ⊙ 14; or ⊙ 4 and loose chromosomes; or 3 pairs and loose chro- mosomes. <i>Rubricalyx</i> shows ⊙ 6 and 4 pairs; or ⊙ 4 and 5 pairs. cf tables II, III, VII, IX, XI. Triple heterozy- gote, breeds true ex- cept for minor fea- tures as bud color. In this hybrid "the amount of somatic pairing ap- pears to be variable in the individual and in- dependent of the ar- rangement taken up during meiosis."

TABLE XII—*Continued*

F ₂ HYBRIDS UNLESS OTHER- WISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(biennis×ru- bricalyx)× ammophila	1929, GATES and SHEF- FIELD	GATES	7 pairs	♀ ♂ cf above. "This is the first case in which unlike linkages have been recorded in reciprocal hybrids"; triple hybrid with 7 pairs "makes it clear that complete pairing cannot be regarded as a sign of the homozy- gous condition."
ammophila× rubricalyx der.	1929, SHEF- FIELD	?	⊙ 6 and 4 pairs	♀ ♂ cf above.
lamarckiana ×suaveo- lens sulfu- rea, and recip.	1929, CLELAND and OEHL- KERS	OEHLKERS	⊙ 12 and 1 pair	♀ cf above. ♂ ⊙ 12 and 1 pair. cf table VI. Complex <i>gaudens.flavens</i> . Occa- sional transfer of <i>fla- vens</i> lethal.
lamarckiana ×suaveo- lens sul- furea	1929, "	"	2 ⊙s 4 and 3 pairs	♀ ♂ cf above. Complex <i>velans.flavens</i> and recip. Exchange of at least three inde- pendent factors.
suaveolens sulfurea× lamarcki- ana	1929, "	"	⊙ 14	♀ ♂ cf above. Complex <i>albicans.ve- lans</i> . No factor ex- change except flower size.
suaveolens sulfurea× lamarcki- ana	1929, "	"	⊙s 6, 8	♀ ♂ cf above. Complex <i>albicans.gau- dens</i> . Factor exchange only in flower size.
lamarckiana cruciata× strigosa	1929, "	"	⊙s 6, 4, and 2 pairs	♀ ⊙ 12 and 1 pair. cf table VI. ♂ ⊙ 14; ⊙ 12 and 1 pair. cf tables VI, IX, XI. Complex <i>velans.strin- gens</i> . Considerable fac- tor exchange.
lamarckiana cruciata× strigosa	1929, "	"	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex <i>deprimens.ve- lans</i> and <i>deprimens.gau- dens</i> . Occasional split in leaf breadth.

TABLE XII—*Continued*

F ₁ HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
lamarckiana cruciata × strigosa	1929, CLELAND and OEHL- KERS	OEHLKERS	⊙ 14	♀ ♂ cf above. Complex <i>gaudens.strin-</i> <i>gens</i> .
suaveolens sulfurea × strigosa	1929, “	“	⊙ 12 and 1 pair	♀ ♂ cf above. Complex <i>albicans.strin-</i> <i>gens</i> . Exchange only in pollen sterility fac- tor.
suaveolens sulfurea × strigosa	1929, “	“	⊙ 12 and 1 pair	♀ ♂ cf above. Complex <i>deprimens fla-</i> <i>vens</i> . No factor ex- change except in flower size.
suaveolens sulfurea × strigosa	1929, “	“	⊙ 4 and 5 pairs	♀ ♂ cf above. Complex <i>flavens.strin-</i> <i>gens</i> . Exchange in at least four independent factors.
suaveolens × cockerelli, and recip.	1929, “	“	⊙ 12 and 1 pair	♀ ♂ cf above. Complex <i>albicans.elon-</i> <i>gans</i> . No factor ex- change according to DEVRIES.
suaveolens × cockerelli, and recip.	1929, “	“	⊙ 12 and 1 pair	♀ ♂ cf above. Complex <i>curtans fla-</i> <i>vens</i> . No factor ex- change.
suaveolens × cockerelli, and recip.	1929, “	“	⊙ 8 and 3 pairs	♀ ♂ cf above. Complex <i>flavens.elon-</i> <i>gans</i> . Occasional factor exchange of four fac- tors.
suaveolens × strigosa	1926, OEHL- KERS	“	⊙ 12 and 1 pair; 7 pairs	♀ ♂ cf above. Complex <i>flavens.strin-</i> <i>gens</i> . F ₁ gave twin hy- brids, <i>albata</i> and <i>flava</i> , former with ⊙ 12 and 1 pair breeding true and latter with 7 pairs segregating many types.

TABLE XII—Continued

F ₁ HYBRIDS UNLESS OTHER- WISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
suaveolens × strigosa	1929, OEHL- KERS	OEHLKERS	⊙ 14; ⊙ 4 and 5 pairs	♀ ♂ cf above. F ₁ gave twin hybrids, <i>albata</i> and <i>flava</i> ; former is constant in breeding behavior (⊙ 14; ⊙ 12 and 1 pair); latter seg- regates many forms (⊙ 4 and 5 pairs; 7 pairs).
biennis × su- veolens	1928, CLELAND 1929, HOEP- PENER and RENNER	DEVRIES ?	⊙ 12 and 1 pair	♀ ⊙s 6 and 8; ⊙ 14; ⊙ 4 and loose chromo- somes; 3 pairs and loose chromosomes. cf table VII, IX, XI. ♂ cf above. "Hybrid is in reality practically or wholly identical with the spe- cies <i>suaveolens</i> and in- distinguishable from it phenotypically. When selfed, it breeds true for this configuration" (14). Complex <i>albicans</i> . <i>flavens</i> .
(suaveolens × pachy- carpa) F ₂	1930, RUDLOFF	?	⊙ 6 and 4 pairs; ⊙s 6 and 4 and 2 pairs	♀ cf above. ♂ ⊙ 14. cf table IX.
suaveolens × pachy- carpa	1930, "	?	⊙ 6 and 2 ⊙s 4	♀ ♂ cf above. Complex <i>flavens.subcur- vans</i> .
muricata × pachy- carpa	1930, "	?	⊙ 14	♀ ♂ cf above. Complex <i>rigens.subcur- vans</i> .
lamarckiana × pachy- carpa	1930, "	?	⊙ 14	♀ ♂ cf above. Complex <i>subcurvans.ve- lans</i> .
pachycarpa × lamarcki- ana	1930, "	?	⊙ 12 and 1 pair	♀ ♂ cf above. Complex <i>augens.velans</i> .
(purpurata × suaveolens) F ₂	1929, "	?	7 pairs	♀ 7 pairs. cf table I. ♂ cf above. Complex <i>purpurata</i> . <i>flavens</i> .

TABLE XII—Continued

F. HYBRIDS UNLESS OTHER- WISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(purpurata × suaveolens) × biennis	1929, RUDLOFF	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex <i>rubens.flavens</i> .
purpurata × cruciata	1929, “	?	⊙ 4 and 5 pairs	♀ cf above. ♂ ? Complex ^h <i>purpurata</i> . <i>flectens</i> .
(purpurata × lamarckiana) F ₂	1929, “	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex ^h <i>purpurata</i> . <i>gaudens</i> .
(purpurata × lamarckiana) × pur- purata	1929, “	?	⊙ 12 and 1 pair	♀ ♂ cf above. Complex ^h <i>purpurata</i> . <i>velans</i> .
hookeri × la- marckiana	1929, HOEP- PENER and RENNER	RENNER	⊙ 4 and 5 pairs; 3 pairs and 2 ⊙s 4	♀ 7 pairs. cf table I. ♂ cf above. Complex ^h <i>hookeri.velu-</i> <i>tina</i> .
hookeri × la- marckiana	1929, HOEP- PENER and RENNER	RENNER	⊙ 10 and 2 pairs (prob- ably)	♀ ♂ cf above. Complex ^h <i>hookeri.gau-</i> <i>dens</i> .
hookeri × sua- veolens	1928, CLELAND 1930, CLELAND and BLAKES- LEE	DEVRIES	⊙ 4 and 5 pairs	♀ ♂ cf above. Complex <i>flavens.hhook-</i> <i>eri</i> .
suaveolens × hookeri	1930, CLELAND and BLAKES- LEE	?	⊙ 14	♀ ♂ cf above. Complex <i>albicans</i> . ^h <i>hookeri</i> .
hookeri × biennis	1930, CLELAND and BLAKES- LEE	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex ^h <i>hookeri.ru-</i> <i>bens</i> .
biennis × hookeri	1928, CLELAND	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex <i>rubens.hhook-</i> <i>eri</i> .
R-biennis × hookeri	1928, CLELAND 1929, HOEP- PENER and RENNER	RENNER RENNER	⊙ 14 com- mon (<i>al-</i> <i>bata</i>); ⊙ 10 and 2 pairs (<i>rubefacta</i>)	♀ ⊙s 6 and 8. cf table VII. ♂ cf above. Complex <i>albicans</i> . ^h <i>hookeri</i> .

TABLE XII—*Continued*

F ₁ HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
chicagoensis × hookeri	1930, CLELAND and BLAKES- LEE	CLELAND and BLAKES- LEE	⊙ 4 and 5 pairs	♀ ⊙ 12 and 1 pair. cf table VI. ♂ cf above. Complex <i>excellens.hookeri</i> .
chicagoensis × biennis	1931, CLELAND	?	⊙ s 6, 8	♀ ♂ cf above. Complex <i>punctulans.rubens</i> ; metacline hy- brid.
biennis×me- tactline	1931, CLELAND	?	⊙ s 6, 8	♀ cf above. ♂ ⊙ s 6, 8. cf table VII. Complex <i>albicans.ru- bens</i> .
chicagoensis × suaveo- lens	1930, CLELAND and BLAKES- LEE	CLELAND and BLAKES- LEE	2 ⊙ 4 and 3 pairs	♀ ♂ cf above. Complex <i>excellens fla- vens</i> .
lamarckiana × chicao- ensis	1930, CLELAND and BLAKES- LEE	CLELAND and BLAKES- LEE	⊙ 6 and 4 pairs	♀ ♂ cf above. Complex <i>velans.excel- lens</i> .
pachycarpa × hookeri	1930, RUDLOFF	?	⊙ 10 and 2 pairs	♀ ♂ cf above. Complex <i>hookeri.au- gens</i> .
biennis× pachy- carpa	1930, RUDLOFF	?	⊙ 14	♀ ♂ cf above. Complex <i>albicans.sub- curvans</i> .
biennis×mu- ricata	1928, CLELAND	DEVRIES	⊙ s 4, 6 and 2 pairs; ⊙ 4 and 5 pairs	♀ ♂ cf above.
biennis×la- marckiana	1930, HÅKANS- SON	?	⊙ s 6, 8	♀ ♂ cf above. Complex <i>albicans.gau- dens(?)</i> .
biennis×la- marckiana	1930, HÅKANS- SON	?	⊙ 14	♀ ♂ cf above. Complex <i>albicans.ve- lans</i> .
biennis sulfu- rea, hanno- ver×cock- erelli	1929, CLELAND and OEHL- KERS	OEHLKERS	⊙ 12 and 1 pair	♀ ? ♂ cf above. Complex <i>albicans.elon- gans</i> . No exchange ex- cept in flower size, to which one or more genes are linked.

TABLE XII—*Continued*

F ₁ HYBRIDS UNLESS OTHER- WISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
<i>lamarckiana</i> × <i>rubri-</i> <i>calyx</i> , Afterglow	1930, EMERSON	?	⊙ 12 and 1 pair	♀ cf above. ♂ ⊙ 8 and 3 pairs. cf table IV. Complex <i>gaudens</i> . mod- ified <i>velans</i> of <i>rubri-</i> <i>calyx</i> . F ₁ has <i>lamarcki-</i> <i>ana</i> growth habits.
<i>lamarckiana</i> × <i>biennis</i> , Chicago	1928, CLELAND 1928, HÅKANSON	DEVRIES ?	⊙ 12 and 1 pair	♀ cf above. ♂ ? Heterogamous (pol- len and egg carry dif- ferent qualities). This cross gives <i>fallax</i> (table VI).
(<i>lamarckiana</i> × <i>rubri-</i> <i>calyx</i> , Af- terglow) F ₁ (selfed)	1930, EMERSON	?	⊙ 12 and 1 pair	♀ ♂ cf above. F ₁ of above cross selfed. Breeds true for bud color, growth habit, and chromosome con- figuration.
<i>lamarckiana</i> × <i>rubri-</i> <i>calyx</i> , Af- terglow	1930, “	?	⊙ 8 and 3 pairs	♀ ♂ cf above. Complex <i>gaudens</i> . <i>lati-</i> <i>frons</i> of <i>rubricalyx</i> (no lethal). F ₁ of this cross resembles <i>lamarckiana</i> in growth habit.
(<i>lamarckiana</i> × <i>rubri-</i> <i>calyx</i> , Af- terglow) F ₁ (selfed)	1930, “	?	7 pairs	♀ ♂ cf above. F ₁ of above cross selfed. Gives two types: (1) F ₁ type with growth hab- its variable, and (2) type homozygous for <i>rubricalyx</i> bud color, bud shape of mut. <i>lati-</i> <i>frons</i> , and growth habit variable. Hybrid with 7 pairs.
<i>lamarckiana</i> × <i>rubri-</i> <i>calyx</i> , Af- terglow	1930, “	?	⊙ 8 and 3 pairs	♀ ♂ cf above. Complex <i>velans</i> . <i>lati-</i> <i>frons</i> . F ₁ has <i>rubri-</i> <i>calyx</i> growth habit.

TABLE XII—*Continued*

F. HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(<i>lamarckiana</i> × <i>rubri-</i> <i>calyx</i> , Af- terglow) F ₁ (selfed)	1930, EMERSON	?	⊙ 8 and 3 pairs; 7 pairs	♀ ♂ cf above. F ₁ of above cross selfed. First configuration seen in two types: <i>rubri-</i> <i>calyx</i> habits and buds and <i>rubricalyx</i> habits and red buds; second configuration seen in two types: <i>latifrons</i> habits and <i>rubricalyx</i> buds, and <i>latifrons</i> hab- its and red buds. Hy- brid with 7 pairs.
mut. <i>recidiva</i> (selfed)	1929, KULKARNI	DAVIS (BART- LETT)	⊙ 14; 7 pairs	♀ ♂ ⊙ 14. cf table IX. Throws twin hybrids. Hybrid with ⊙ 14 has complex aB and resem- bles parents, while aa complex is lethal. Hy- brid with 7 pairs has complex BB and is gray non-flowering. <i>Re-</i> <i>cidiva</i> is mutant of <i>pra-</i> <i>tincola</i> , table VI.
<i>pratincola</i> E mut. "for- mosa" × <i>pratincola</i> C	1929, "	DAVIS	⊙ 12 and 1 pair	♀ ⊙ 14. cf table IX. ♂ ⊙ 14. cf table IX.
mut. <i>recidiva</i> × mut. <i>si-</i> <i>mulans</i>	1929, "	DAVIS (BART- LETT)	⊙ 14; ⊙ 10 and 2 pairs	♀ ⊙ 14, cf table IX. ♂ ⊙ 14; ⊙ 12 and 1 pair. cf tables VI, IX, XI. Hybrid with ⊙ 14 has complex aB (<i>recidiva</i> with red buds). The aa complex is lethal. Hy- brid with ⊙ 10 and 2 pairs is gray form with complex BB.
mut. <i>simulans</i> × mut. <i>reci-</i> <i>diva</i>	1929, "	DAVIS (BART- LETT)	⊙ 10 and 2 pairs; ⊙ 14	♀ ♂ cf above. Hybrid with ⊙ 10 and 2 pairs has complex aa. Hybrid with ⊙ 14 has complex aB. <i>Simulans</i> a is said to be different from <i>recidiva</i> a.

TABLE XII—*Continued*

F ₁ HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
eriensis × ammophila	1929, SHEP-FIELD	GATES	⊙ 12 and 1 pair	♀ ⊙ 14. cf table IX. ♂ ⊙ 14 less common; ⊙ 12 and 1 pair. Latter is common and "like pollen parent" (GATES). cf tables VI, IX, XI.
ammophila × eriensis	1929, "	"	⊙ 14	♀ ♂ cf. above. Resembles pollen parent (GATES).
ammophila × novae-scotiae	1929, "	"	⊙ 14	♀ cf above. ♂ ⊙ 14; ♂s 6, 8 cf tables VII, IX, XI.
eriensis × rubricalyx	1929, "	"	⊙ 12 and 1 pair	♀ cf above. ♂ ⊙ 6 and 4 pairs; ⊙ 4 and 5 pairs. cf tables II, III, XI.
rubricalyx × novae-scotiae	1929, "	"	⊙ 12 and 1 pair	♀ ♂ cf above.
franciscana × franciscana sulfurea	1931, EMERSON	DAVIS	⊙ 4 and 5 pairs	♀ ⊙ 4 and 5 pairs; 7 pairs; chain of 4 or more. cf tables I, II, XI. ♂ ⊙ 12 and 1 pair; 7 pairs; cf tables I, VI, XI. One plant of pedigree 58-1 with red buds gave the ⊙ 4 and 5 pairs. Complex <i>franciscana. s-d-franciscans</i> .
franciscana sulfurea × franciscana	1931, "	"	7 pairs	♀ ♂ cf above. One plant of pedigree 59-1 with red buds gave 7 pairs. Complex <i>franciscans.s-d-franciscans</i> .
(franciscana × franciscana sulfurea) F ₂	1931, "	"	⊙ 4 and 5 pairs; 7 pairs	♀ ♂ cf above. In this F ₂ of 58-1 two plants showed ⊙ 4 and 5 pairs and one plant showed 7 pairs.

TABLE XII—*Continued*

F ₁ HYBRIDS UNLESS OTHERWISE INDICATED	WHEN AND BY WHOM REPORTED	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATIONS OF HYBRID	PARENTAL CHROMOSOME CONFIGURATIONS, ETC.
(franciscana × franciscana sulfurea)F ₂	1931, EMERSON	DAVIS	7 pairs; and 1 haploid	♀ ♂ cf above. Of ten plants with red budded cones, one (287-1) of 58-1, was haploid; others (287-3, 5, 7, 10, 31 and 288-1, 4, 81) had each 7 pairs.
(franciscana × franciscana sulfurea)F ₂	1931, “	“	⊙ 10 and 2 pairs; 2 pairs, tri- valent group and chain of 7 or 8	♀ ♂ cf above. Of four plants with green bud-cones (288- 5, 20, 71, 60) of 58-1, three showed ⊙ 10 and 2 pairs. Complex <i>sulfurea franciscana s-d franciscana</i> .
franciscana sulfurea × latifrons	1928, CLELAND	?	⊙ 6 and 4 pairs	♀ cf above. ♂ 7 pairs. cf table I.

Table XIII consists of twenty-five hybrids grouped separately because they are triple heterozygotes especially prepared genetically by Professor SHULL. They consist of one group of twenty-one *lamarckiana* types and a smaller group of four non-*lamarckiana* types.

Nineteen of the twenty-one *lamarckiana* types have shown one or more configurations also seen in *lamarckiana*. The other two of this group have thus far shown only 4 pairs and ⊙ 6. Two of these twenty-one *lamarckiana* types have shown two types of configurations in the same bud, one case in cells four sections apart on the slide, and another in adjacent cells of the same loculus, respectively. Such a condition would make it difficult to assume that any particular type of closed circle is associated with a particular phenotypical constitution.

Of the non-*lamarckiana* group, all four F₁ offspring show configurations resembling one or both parents. One configuration of this group consists of 7 pairs which indicates that complete pairing is possible in spite of triple heterozygosity.

The data presented in tables XII and XIII offer additional evidence in favor of the hypothesis that chromosomal configurations in the *Oenotheras* are inherited characteristics due to genic action.

TABLE XIII

CHROMOSOME CONFIGURATIONS OF OENOTHERA TRIPLE HETEROZYGOTES
ESPECIALLY MADE UP BY SHULL

LEGEND FOR ABBREVIATIONS:

ang, angustifolia	R, rubricalyx (dominant)
ammop, ammophila	rc, rubricalyx
bull, bullata	sp, suplena
br, brevistylis	sten, stenophylla
ery, erythrina	A, aurata (flower color)
f, franciscana	S, sulfur flower color
fun, funifolia	V, vetaurea ("old gold" flower color)
lam, lamarckiana	Y, yellow flower color
oi, "outside in"	o, long style
p, pervirens	

FORMATION OF HYBRID	STUDIED BY ILICK IN	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATION	PARENTAL CHROMOSOME CONFIGURATIONS
<i>Lamarckiana</i> types: Since these parents are all <i>lamarckiana</i> types, they might be expected to show one or more of its configurations, 7 pairs (?); 3 pairs and chain; 2 pairs and \odot 10; 1 pair and \odot 12; chain of 14.				
lam.Y.o.Xbull. Y.br.	1930	SHULL	\odot 12 and 1 pair (from different plants)	♀ ♂ <i>lamarckiana</i> types.
lam.Y.o.XY.br.	1930	"	\odot 12 and 1 pair	♀ ♂ <i>lamarckiana</i> types.
lam.Y.o.Xlam.V.oi.	1930	"	\odot 10 and 2 pairs	♀ ♂ <i>lamarckiana</i> types.
lam.Y.Xrc.A.oi.	1930	"	\odot 12 and 1 pair (from different plants)	♀ <i>lamarckiana</i> type. ♂ Derivative of <i>franciscana</i> <i>sulfurea</i> which shows 7 pairs; \odot 12 and 1 pair.
lam.S.o.XY.oi.	1930	"	\odot 10 and 2 pairs	♀ ♂ <i>lamarckiana</i> types.
lam.A.Xlam.Y.oi.	1930	"	\odot 8 and 3 pairs (from different plants)	♀ ♂ <i>lamarckiana</i> types.
p.Y.o.Xrc.A.oi.	1930	"	\odot 6 and 4 pairs (from one plant) or \odot 12 and 1 pair; or \odot 6 and 4 pairs (from different plants)	♀ <i>pervirens</i> (<i>lamarckiana</i> type). ♂ cf above.
p.Y.o.Xp.V.oi.	1930	"	\odot 6 and 4 pairs	♀ ♂ <i>pervirens</i> .

TABLE XIII—*Continued*

FORMATION OF HYBRID	STUDIED BY ILICK IN	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATION	PARENTAL CHROMOSOME CONFIGURATIONS
p.V.o.×Y.oi.	1930	SHULL	⊙ 12 and 1 pair, ⊙ 6 and 4 pairs (in one plant), and ⊙ 12 and 1 pair (in one plant)	♀ <i>pervirens</i> (<i>lamarckiana</i> type). ♂ <i>lamarckiana</i> type.
p.V.o.×bull.Y.br.	1929	"	⊙ 12 and 1 pair (from two different plants)	♀ ♂ <i>pervirens</i> , selfed.
p.V.o.×fun.Y.br.	1929	"	⊙ 12 and 1 pair, and ⊙ 14 (in same bud and four sections apart on slide)	♀ ♂ <i>pervirens</i> , selfed.
p.V.o.×rc.S.V. br.sp.	1929	"	⊙ 12 and 1 pair (in one plant)	♀ ♂ <i>pervirens</i> , selfed.
p.sp.V.×bull.Y.br.	1929	"	⊙ 12 and 1 pair (in one plant)	♀ ♂ <i>pervirens</i> , selfed.
ang.Y.o.×bull.Y. br.	1930	"	⊙ 12 and 1 pair (in two different plants)	♀ <i>lamarckiana</i> type. ♂ <i>pervirens</i> (<i>lamarckiana</i> type).
ang.Y.o.×V.br.	1930	"	⊙ 12 and 1 pair	♀ ♂ <i>lamarckiana</i> types.
ang.Y.o.×rc.A.oi.	1930	"	⊙ 12 and 1 pair (in two different plants)	♀ <i>lamarckiana</i> type. ♂ Derivative of <i>franciscana sulfurea</i> which shows ⊙ 12 and 1 pair, or 7 pairs.
sten.Y.o.×V.br.	1930	"	⊙ 12 and 1 pair (in different plants)	♀ ♂ <i>lamarckiana</i> types.
ammop.Y.o.×rc. A.oi.	1930	"	⊙ 8 and 3 pairs	♀ <i>lamarckiana</i> type. ♂ cf above.
ammop.×rc.A.oi.	1930	"	⊙ 6 and 4 pairs	♀ <i>lamarckiana</i> type. ♂ cf above.
rc.Y.o.×R.V.br.	1930	"	⊙ 10 and 2 pairs (in two different plants); or ⊙ 12 and 1 pair and ⊙ 14 (in adjacent cells of same locus)	♀ ♂ <i>lamarckiana</i> types.
rc.S.o.×V.br.	1930	"	⊙ 12 and 1 pair (in two different plants)	♀ ♂ <i>lamarckiana</i> types.

TABLE XIII—Continued

FORMATION OF HYBRID	STUDIED BY ILICK IN	SOURCE OF MATERIAL	CHROMOSOME CONFIGURATION	PARENTAL CHROMOSOME CONFIGURATIONS
Triple heterozygotes of non-lamarckiana types				
f.Y.o.×rc.A.oi.	1930	SHULL	⊙ 4 and 5 pairs	♀ ⊙ 4 and 5 pairs; 7 pairs; chain of 4 or more. cf tables I, II, XI. ♂ Derivative of <i>franciscana sulfurea</i> which shows ⊙ 12 and 1 pair; 7 pairs.
f.S.o.×lam.V.oi.	1930	"	7 pairs	♀ ⊙ 12 and 1 pair; 7 pairs. cf tables I, VI, XI. ♂ ? F ₁ is a triple heterozygote with 7 pairs.
ery.Y.o.×rc.V.br.	1930	"	⊙ 6 and 4 pairs (common); ⊙ 8 and 3 pairs	♀ ⊙ 6 and 4 pairs. cf table III. ♂ ?
biennis L.×rc.A.oi.	1930	"	⊙ 10 and 2 pairs and ⊙s 6, 8 (in different plants)	♀ ⊙ 14; ⊙s 6, 8; ⊙ 4 and loose chromosomes; 3 pairs and loose chromosomes. cf tables VII, IX, XI. ♂ cf above.

Discussion

In regard to the *Oenothera* problem the two following facts, from cytological and genetical studies respectively, should be kept in mind: (1) the presence of closed circles which are only relatively constant for any one species; and (2) the genetical phenomena whereby great numbers of old parental types or combinations and relatively few new combinations often appear in the offspring of crosses, indicating some kind of linkage or determinate assortment.

The question arises, do closed circles in the *Oenotheras* bear any relation to their peculiar genetical behavior? Or in other words, is chromosome cohesion the basis of this linkage or determinate assortment of factors? The writer believes that any attempt to explain these two phenomena should be kept separate since evidence is available which permits such a separation.

DARLINGTON (18) recognizes that "in spite of the hybridity of the ring, pairing is extraordinarily regular. Many homozygous plants which normally form bivalent rings (*Primula*, *Matthiola*, *O. desertens*, HÅKANSSON 38) also produce, by failure of the chiasma in one arm of the paired chromosomes, a rod united by a single chiasma;" nevertheless he holds, as a general rule, that "ring forming segregates of *Oenothera* crosses are hybrids, while bivalent forming segregates are homozygous."

By carefully studying these thirteen tables it is to be noted that complete pairing of the chromosomes apparently takes place equally as well among recognized heterozygous individuals as among those which are said to be homozygous. This would seem to rule out homozygosity and heterozygosity as factors relating to the formation of bivalent chromosome pairs or closed circles.

Table XI lists twenty-nine individuals, each of which has shown *two or more chromosomal configurations*. The complexes for eleven of these are known and are given in each instance, one complex for each individual. In other words, two or more chromosomal configurations have been observed in each of eleven *Oenotheras*, each of which had only one known complex. In table XII there are four crosses, the F_1 of each showing two or more different types of chromosomal configuration when only one complex is recognized. *Lamarckiana* \times *grandiflora* gives an F_1 with \odot s 10 and 4 (CLELAND and OEHLKERS) and 7 pairs (GERHARD) with the complex *truncans. velans* in each instance. *Lamarckiana* \times *grandiflora* also gives an F_1 with \odot 14 (CLELAND and OEHLKERS) and \odot 10 and 2 pairs (GERHARD) with the complex *acuens.gaudens* in each case. *Grandiflora* \times *lamarckiana* gives an F_1 with \odot s 6, 4 and 2 pairs (GERHARD) and *lamarckiana* \times *grandiflora* gives an F_1 with \odot 4 and 5 pairs (GERHARD), in both of which there is the complex *acuens.velans*. And *suaveolens* \times *strigosa* gives an F_1 with \odot 4 and 5 pairs (CLELAND and OEHLKERS) and \odot 12 and 1 pair or 7 pairs (OEHLKERS) with the complex *flavens.stringens* in each instance.

Additional data bearing on this question are from *pervirens*, selfed and a *lamarckiana*-type in table XIII. The complex for *pervirens* is doubtless *velans.gaudens*; the complex for the other is not known. *Pervirens*, selfed has shown \odot 14, and \odot 12 and 1 pair in cells on the

same slide four sections apart, and the *lamarckiana*-type individual has shown \odot 14 and \odot 12 and 1 pair in adjacent cells of the same locus. *Germanica* from table XI should also be mentioned in this connection. Its complex is not available. It has shown \odot 14, \odot 12 and 1 pair, and \odot 10 and 2 pairs, all "in one and the same slide."²

This evidence would seem to indicate that the complexes, as such, bear no particular relation to the types of configurations in particular species.

It has been suggested by some investigators that the production of numerous old parental combinations or apparent linkages is due to chromosome cohesion in the form of closed circles, and that the chromosomes are arranged in a determinate fashion. Special studies are now in progress which will definitely decide the question of a determinate or indeterminate arrangement of the chromosomes in closed circles of the *Oenotheras*. If the arrangement should be found to be indeterminate (SHULL 60), we can conclude that closed circles are not related to the peculiar genetical behavior of the *Oenotheras*, and that some other explanation must be found to account for the unusual genetical behavior. Some preliminary evidence has been given (ILLICK 42) which indicates an indeterminate arrangement of the chromosomes. Such an arrangement would permit segregation in spite of closed circles. The case of *grandiflora* Ait., a "complex heterozygote," has already been mentioned in table IX as one which has thus far shown three types of configurations (\odot 14, \odot 12 and 1 pair, and 7 pairs) and at the same time has shown segregation of two forms (the third being non-viable) in each generation with no apparent regard to the type of chromosomal configuration. HOEPFENER and RENNER (40) hold that the principle that chromosome cohesion is at the basis of extensive linkage in *Oenothera* should be viewed with reserve, inasmuch as the flower-size factor behaves independently of the presence or absence of large circles.

The idea of segmental interchange has been presented to explain the peculiar genetical behavior of the *Oenotheras*. Those who offer

² In regard to the three configurations in *germanica*, LELIVELD claims they are the consequence of fixation, but she does not state which should be the normal configuration. I have disregarded this view throughout this study since it does not seem likely that the same fixation should cause such differences among the chromosomes of the same bud.

this hypothesis based upon work with *Datura* and *Drosophila* (BLAKESLEE 1, DARLINGTON 17, 18, and MÜLLER 48) also hold that closed circles result from such interchange between non-homologous chromosomes. SHEFFIELD (55) does not believe that there is any real parallelism between *Oenothera* and *Datura*, and that DARLINGTON's theory concerning the *Oenothera* situation, based on a comparison with chromosome linkage in *Datura*, which assumes that the ring formation of the chromosomes results from segmental interchange between non-homologous chromosomes, has no advantage.

MÜLLER (48) has presented evidence, based upon studies on *Drosophila* involving the second and third chromosomes, which indicates a determinate rather than a random action on the part of the characters studied, and consequently resulting in fewer new combinations than would be expected on the basis of independent assortment. It is further suggested that the absence of closed circles in *Drosophila* is due to the fact that it is a dioecious individual and therefore less likely to show such a condition than a monoecious form. *Oenothera* is monoecious and therefore, it is claimed, closed circles would be expected more often. But if reference is made to table XIII, there will be found a number of *Oenothera* crosses especially prepared by Professor SHULL. These are *not selfed*, as is true for many monoecious individuals, and thus simulate *Drosophila* crosses. Yet closed circles continue to be found in the F_1 of such crosses. The Copepod (*Diaptomus castor*) is said to have a \odot 6 and 14 pairs of chromosomes (MATSCHECK 47) and is a dioecious individual. It would appear therefore that the dioecious and monoecious conditions play no particular part in the relative absence or abundance of closed circles of chromosomes.

It should be noted that the data presented in connection with each of these thirteen tables permits the assumption that genic factors are involved, and that chromosome cohesion is a heritable trait and bears no relation to the genetical behavior of the individual in other respects. This suggestion was first made by SHULL and later by ILLICK (41). CLELAND (14) claims that in the study of eighteen true reciprocal hybrids, "in every case the reciprocals have been found to have identical chromosome configurations." This condition might

well be expected on the assumption that chromosomal configurations are heritable traits. SHEFFIELD (55) in a recent paper also suggests that chromosome linkage may be inherited as a genetic character, and that an examination of all available data shows nothing contrary to such a possibility.

Segmental interchange eventually may be shown to be the factor involved in the production of large numbers of parental types and the few new combinations in the *Oenotheras*, as has been found for *Drosophila*, and without assuming it to be a direct causal factor in the formation of closed circles. The relation that segmental interchange would doubtless have in connection with the formation of closed circles according to my hypothesis is an indirect one. The genes that are assumed to be the direct causal factors could, by segmental interchange, be transferred to other chromosomes, thus making possible other types of chromosomal configurations in the succeeding generation.

Summary and conclusions

1. The evidence from tables I to XI indicates that chromosomal configurations are only relatively constant and not necessarily characteristic for any particular species.

2. Three forms, *germanica* (table XI), *pervirens*, selfed (a triple heterozygote, table XIII), and a triple heterozygous *lamarckiana*-type (table XIII) have each been observed to show two or more different configurations. In the first were seen three different configurations in the same slide (\odot 14, \odot 12 and 1 pair, and \odot 10 and 2 pairs); in the second, two different configurations were observed in two cells of the same bud four sections apart (\odot 14 and \odot 12 and 1 pair); and in the third form there were seen two different configurations in adjacent cells of the same locus (\odot 14 and \odot 12 and 1 pair). Twenty-seven other species, mutants, etc., have also shown two or more chromosomal configurations.

3. Whenever variable chromosomal configurations have occurred there have been recorded no particular phenotypical changes with which they might be associated.

4. The presence of seven complete pairs of chromosomes in rec-

ognized heterozygotes indicates that heterozygosity plays no particular part in the formation of closed circles.

5. The evidence seems to indicate that the complexes, as such, as recorded in tables XI, XII, and XIII, bear no special relation to particular chromosomal configurations.

6. It is therefore assumed that the formation of closed circles in the *Oenotheras* bears no particular relation to their observed genetical behavior. An indeterminate arrangement of the chromosomes in closed circles would offer additional and decisive evidence in support of this assumption.

7. The evidence presented permits the assumption that closed circles are due to genic action as are phenotypical characters and are therefore heritable traits.

8. The assumption that segmental interchange is the factor involved in the production of the unusual number of old parental combinations and the few new combinations is believed to be compatible with the assumption that cohesion of chromosomes to form closed circles is due to genic action.

9. These two phenomena, chromosome cohesion to form closed circles and the unusual genetical behavior in the *Oenotheras*, are therefore assumed to bear no direct relation to each other.

10. Segmental interchange might possibly have an indirect relation to the formation of closed circles, in that the genes assumed to be the direct causal factors could, by segmental interchange, be transferred to other chromosomes, making possible other types of configurations in the succeeding generation.

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ROOT SYSTEM OF QUERCUS MACROCARPA IN RELATION TO THE INVASION OF PRAIRIE

J. E. WEAVER AND JOSEPH KRAMER

(WITH TEN FIGURES)

Introduction

Invasion of trees into grassland is primarily a phenomenon of plant competition. It involves the relation of the invaded and the invader to light and other atmospheric factors of the habitat, both physical and biotic. But perhaps even to a greater degree the edaphic factors, and especially water in dry climates, are concerned. Consequently to understand the reasons for plant distribution and the changes resulting from natural successions, a knowledge of the parts of the plants underground and of the interrelations of the root systems is of fundamental importance.

The great tension zone or ecotone between the deciduous forest formation of the eastern United States and the grassland formation that centers west of the Missouri River occurs, in part, in eastern Nebraska. It offers a convenient and fascinating field for the study of the age-long struggle between grassland and woodland for possession of the territory bordering the Missouri River. Here the many factors of soil and climate, controlled by limited humidity above ground and scarcity of water beneath, offer conditions under which it is just possible for sturdy pioneer trees to ecize but never to develop to the stature of their kind under more congenial conditions of growth. Against their invasion *Andropogon* and other grasses compete most severely and in the main successfully.

Much work has been done on the root habits of the prairie vegetation; some studies have been made of the underground parts of the bordering chaparral; but little is known of the root relations of deciduous forest trees, except in the seedling stage of growth. To understand the problem of the invasion of trees into grassland, some knowledge of the soil and climate and of the character of the grassland is indispensable.

SOILS AND CLIMATE

The soils of eastern Nebraska, where these studies were made, are rather diverse in character. They include the mellow loess soils immediately bordering the Missouri River, and also those of the drift hills and of the alluvial plains and terraces. Whether the Wabash silt loam of the valleys or the Carrington silt loam of the uplands or other soil types are concerned, they all agree in having great depth and a constantly moist subsoil. After studying the root habits of different types of plants in a wide range of habitats, it has been found that various types of soils may influence the environment in a very similar manner. The ecological significance of various soil types is related to their ability to supply water and the necessary nutrients to the vegetation. The cover of grassland, for example, is very similar in species, structure, and manner of growth throughout much of eastern Nebraska. The various types of prairie that do occur are clearly related to water content of soil, but only obscurely, if at all, to soil type (cf. 18).

In general, the soils are deep, fertile, fine-textured loams of high water-holding capacity and are circumneutral in reaction. They readily absorb water and the subsoil is always moist to great depths. The mean annual precipitation varies from 28 to 33 inches, nearly 80 per cent of it falling during the growing season. Periods of drought of 15-30 days' duration are likely to occur at any time, and especially after midsummer.

Studies extending over a period of 12 years have been pursued at Lincoln, Nebraska, where the mean annual precipitation is 28 inches (30). It has been shown that the available water content in the surface 6 inches of upland soil, which supports a growth of the climax prairie vegetation, was reduced to less than 5 per cent at one or more times nearly every year; but only twice during this period was it reduced to the hygroscopic coefficient. In the second 6-inch level, available water content exceeded 5 per cent three-fourths of the time but was frequently reduced to 2-3 per cent. At no time, however, was the water available for plant growth entirely exhausted. In the second, third, and fourth foot, where water content was less variable, the available supply usually ranged between 5 and 15 per

cent. The maximum was 21 per cent and a few times the minimum fell to 1-3 per cent. In lowland prairie the available water content was 3-10 per cent greater in the surface foot and 5-11 per cent in excess of that of the upland in the deeper soil.

Under an increasing rainfall eastward (maximum 33 inches in Nebraska), the water content has been found to be rather consistently somewhat higher. This is indicated by the greater abundance and wider distribution of the less xeric prairie species, by the growth of chaparral, and by the appearance of the forest. Intermittent determinations of the water content, often during root examinations where excavations 8-22 feet in depth were made, have shown that the soil below 4 feet is also rather moist. It is upon the direct supply of water from rainfall that invading upland forest trees depend. They are not furnished with moisture from the water table (7).

Not only is the soil relatively dry when compared with that of the main body of the deciduous forest, but also the air has a great power of desiccation. It is a region with much sunshine, high summer temperatures, high evaporation, much wind movement, and relatively low humidity. During March, April, and May there is approximately 60 per cent sunshine; but June, July, and August have 72 per cent or more. Average day air temperatures sometimes reach 90°F., and maximum air temperatures sometimes reach or exceed 100°F. More usually the average day temperatures are from 75° to 85°. The average night temperatures are 10° or more lower. Evaporation rarely falls below an average weekly loss of 10 cc. per day and is more usually 20-30 cc. During periods of drought, however, it sometimes reaches 40-55 cc. Wind movement is rather constant and often high, and is an important factor in promoting water loss. The average day humidity ranges between 50 and 80 per cent during years of greater rainfall but falls frequently to 40-50 per cent during drier years. The average night humidity is frequently 20 per cent higher. These combined conditions promote a high rate of transpiration and great evaporation from the surface soil. These water losses have been found to average nearly a pound of water per square foot of soil surface per day (28). Thus water content in the first 6-inch layer of soil varies widely and rapidly, often 10 per cent or more during a single week.

THE PRAIRIE

The grasslands, before they were broken, formed an almost uninterrupted cover over most of the area. The dominant species of lowlands is the big bluestem (*Andropogon furcatus*). This grass is distinctly a sod former, owing to extensive propagation by rhizomes. It grows throughout the entire warm season and forms dense stands 6 to 10 feet in height. Indian grass (*Andropogon nutans*), tall panic grass (*Panicum virgatum*), and nodding wild rye (*Elymus canadensis*) are the principal grasses accompanying it. They have rather similar habits and stature. Near the forest border, owing to a greater supply of water, this type of prairie extends far up the slopes and often entirely over the hills. The grasses are intermixed with many legumes, composites, and other forbs, which are, however, always of minor importance. Although only 12-20 per cent of the soil surface is actually covered, no large spaces are left unoccupied; and the foliage is so dense that the apparent cover is nearly always 90 to 100 per cent (27).

The soil is thoroughly occupied by roots. The really magnificent network of the roots of the grasses completely threads the soil to depths varying from 5 to 10 feet. In the surface especially they form a dense sod. Most of the forbs absorb at even greater depths than the grasses; many extend their root systems to 12-16 feet and some much deeper. The soil is so thoroughly occupied that invasion, even by the roots of trees, seems impossible. The root systems are layered, so that the areas of water control do not too greatly overlap and become too severely competitive.

Smaller areas bordering the forest are held by upland types of grassland. Chief among these is the little bluestem (*Andropogon scoparius*) type. This species ordinarily forms an interrupted sod, the mats or tufts being so dense that few other plants can invade. Accompanying grasses (*A. furcatus*, *Poa pratensis*, etc.) and forbs grow between the mats. Ordinarily only 12-20 per cent of the soil surface is covered by the bases of the plants, but the tops spread so widely that the apparent cover is 80-100 per cent (31). Although the plants in this type of prairie are not so tall (0.5-4 feet) nor the vegetation so dense, yet under ground the space is even more thoroughly occupied. The roots of the grasses are finer and have more

and longer branches, undoubtedly an adaptation to secure sufficient water from these drier uplands. The general level of the mat of grass roots scarcely exceeds 3-5 feet, but the deeper soil is well filled with the more extensive roots of numerous accompanying forbs. Many of these are regularly 8-12 feet deep and some 16 to over 22 feet.

It is with such dense, deeply rooted vegetation that an invading species must compete. The invader must not only endure the shade of the grasses, which is often considerable and not infrequently fatal, but also must wrest from the soil the rather scant water supplies in competition with a wonderfully developed system of absorbing organs. Frequently such invasion is accomplished by shrubs that fringe the forest, and the territory thus gained is later appropriated by the trees.

INVASION BY CHAPARRAL

The more protected ravines of the prairie frequently contain one or more species of shrubs that constitute the marginal chaparral of the deciduous forest. With an increase of soil moisture as the forest margin is approached, these shrubby plants not only become greater in number of species and stature but also they migrate up the slopes and over the hilltops and often form a more or less continuous, narrow border a few rods to a few miles in width between grassland and forest (3). Of the numerous species concerned, the three most important are smooth sumac (*Rhus glabra*), coralberry (*Symphoricarpos symphoricarpos*), and hazelnut (*Corylus americana*). Where they are found in common their zonal distribution on slopes shows *Rhus* to be the most xeric. It is also found farthest from the forest margins and is not very tolerant of shade. *Corylus* is the most shade-enduring and does not pioneer far ahead of the forest border. *Symphoricarpos* is somewhat intermediate.

Rhus glabra spreads into grassland by means of very long underground stems, some of which give rise to new shoots 20 to 30 feet from the parent plant. These stems extend outward from the established area usually at depths of only 3-12 inches. The main roots from old plants extend downward and outward under the grass sod, some finally reaching depths of 7-8 feet; but throughout their course they give rise to numerous branches. Many of these, and sometimes the main roots as well, turn obliquely upward and thread the sur-

face soil with a vast network of absorbing laterals (25). Thus the roots compete severely with those of the grasses for the water which supplies a growing system of shoots. These shoots ultimately coalesce into a closed canopy above the grasses, which finally disappear as a result of shading.

Symphoricarpos symphoricarpos grows in clumps to a height of only 2 to 5 feet. Its shade is frequently so dense as to exclude even the very tolerant *Poa pratensis*, although this and other grasses may occur between the clumps. The root system is usually only 4-6 feet deep, but lack of linear extent is amply compensated by a well developed absorbing surface. The finest rooted grasses alone excel this species in its thorough occupancy of the soil. The area is extended under ground by efficient rhizomes, and above ground runners play a similar but less effective part. The chief means of invasion are shading and establishment of new plants among the invaders by vegetative propagation.

Corylus americana spreads by means of large, woody, underground parts 4-6 inches below the surface of the soil. These give rise to new shoots 1 to 2 feet beyond the parent plant. An abundance of new roots also arise from the rhizomes. Some of the smaller roots run vertically toward the surface and branch profusely into very fine ultimate laterals, for *Corylus*, like the preceding shrubs, is a strong competitor of the grasses for the water in the surface soil. Competition for water is keen at all levels to which the grasses penetrate, the roots of *Corylus* usually extending deeper than those of the grasses. Above ground the grasses disappear in the shade of the overhanging branches which lean outward 1 to 3 feet. In this marginal territory, the prairie grasses first cease to form seed and then die. Often new shoots of *Corylus* spring from rhizomes that have extended well into the marginal area. The additional shade cast by these increases the handicap.

The intrusion of the shrubs is by mass invasion. As new plants invade by rhizomes, a mulch of leaves is held among the stems. This follows closely upon the periphery of the advancing shrubs and is very effective in further handicapping the growth of the already weakened plants of the prairie. An advance of only 2 or 3 feet may

require 3 to 5 years, so delicate is the balance between invaded and invader (8).

The occupation of grassland by forest is often attained only as a result of changes in the habitat brought about by the shrubs. But in many places the bur oak is invading the grassland without the aid of the shrubs. To understand the phenomenon of successful invasion of chaparral or grassland by trees, a knowledge of the root systems of trees is of primary importance.

ROOT SYSTEMS OF TREES

Very little work has been done in America on the root systems of mature trees. In Europe this study has been pursued rather vigorously, especially in Germany, Finland, Russia, and adjacent countries. Most of these studies have centered about coniferous species and much less attention has been given to the trees of deciduous forest. Some of these findings will be discussed later, but a complete bibliography need not be given since such studies have only recently been well summarized. An excellent résumé of the work of European investigators is given by BÜSGEN and MÜNCH (6), and a much more elaborate one by LAITAKARI (14), in his monograph on the root system of *Pinus sylvestris*. He reviews the European investigations from the time of HARTIG (1851) and also includes some American work. More recently STEVENS (22) has given a historical review of both European and American investigations on root growth and root competition of trees.

It is gratifying to note that American foresters are beginning to realize that the major problems of silvicultural practice are intimately related to root growth. Only a beginning has been made, however, and the study of the root systems of trees is at best almost a virgin field in America. The great amount of labor involved in the excavation of even a single mature root system has probably been the principal deterrent factor to such investigations (fig. 1).

Some of the best work in the past ten years, the results of which offer immediate application, has been done with the root systems of fruit trees. In some cases the entire soil mass penetrated by the roots was removed in 50 cm. cubes. For each tree excavated over

60 tons of soil was thus handled. The roots from each cube were weighed and graded. This was supplemented by excavating the larger roots and as much fiber as possible, after careful measurement, and arranging and photographing them in their natural position when all had been removed from the soil (21).



FIG. 1.—Portion of root system of *Ulmus americana* along an eroding ravine; such trees occur on upland soil along the Missouri River and do not necessarily depend upon the roots reaching water table for constant supply of moisture.

The growth of roots of seedlings of five species of deciduous trees has been studied in eastern Nebraska on three classes of sites. The form of the root system appeared to be hereditary with the species and was more or less closely correlated with the water content of the soil in the location naturally occupied by that species (12).

GURSKY (10) has shown that even related species of mature trees may differ in their root habits, not only as to penetration into the soil but also as to root type. For example, *Fraxinus excelsior* has the generalized type of root system while *F. pennsylvanica* differs sharply in lacking the vertical taproots.

Although the root habits of a tree are governed, first of all, by the hereditary growth characters of the species, they are often quite as much the product of environment. Indeed, the impression has been gained by certain investigators that in some cases the root form is an expression of soil environment rather than that of inherent tendencies (24).

The senior writer has found that competition of grasses has a pronounced effect, not only upon the growth of the seedlings but also upon the form of the root system. Three-year-old trees of *Gleditsia triacanthos*, *Acer negundo*, *Fraxinus lanceolata*, and *Ulmus americana* were examined. The dwarfed plants that had grown in competition with the grasses had a relatively diminutive taproot system with few long branches. Those about which the grasses were kept closely clipped were more extensive and somewhat symmetrical in shape. Where the sod had been removed to a distance of 6 inches on both sides of the trees along another row, the root system was very asymmetrical and 5-9 feet deep. The widely spreading branches showed a marked preference for the mulched area, the maximum lateral spread (3-7 feet) always being found in it and not in the sod on either side (8).

According to LUNCZ (16), experimental evidence shows that variations in the root system of the same kind of tree are often greater in different soils than are those of different kinds of trees in the same type of soil. He concludes that the tree itself is not in the main responsible for the development of its root habit, but that this is determined by the composition of the soil and subsoil, nutrients, air, water, competition between root systems, etc. It must accommodate itself to conditions occurring during its life.

Other investigations might be cited, but enough has been given to indicate the need of knowledge of the soil and of the general competitive conditions under which the bur oaks to be described have developed.

Investigation

THE OAK FOREST AND THE SOIL PROFILE

The Carrington silt loam from which the root systems were excavated is a typical prairie soil. It has been occupied by only one or possibly two generations of trees. These have migrated on to the

uplands from the valleys along streams where they were protected from prairie fires. Most of this migration and invasion of prairie has occurred in the 70 years since the early settlement of eastern Nebraska and the cessation of prairie fires. Three lines of evidence substantiate this fact. Pioneers are still living who well remember that the trees existed only as marginal forests in sheltered places, largely along streams. There are no very old trees found standing today nor mounds or pits to mark their former abode. Finally, the soil is scarcely modified, except in its very surface, from that of the profile of typical prairie soil.

The bur oak forest where the excavations were made is typical of those of the forest margin in eastern Nebraska (fig. 2). It consisted of a pure open stand. The trees were mostly about 50 to 65 years old, 30-40 feet tall and 12-18 inches in diameter near the base. They were rather widely spaced (10-40 feet). The individuals had well developed crowns with large branches and stout, somewhat sparse branchlets. About half of the ground was carpeted with *Poa pratensis*, the shade and leaf litter elsewhere being too deep for this grass. *Corylus americana*, *Symphoricarpos symphoricarpos*, *Rhus glabra*, and *Cornus asperifolia* were the characteristic shrubs, although *Rhamnus lanceolata*, *Sambucus canadensis*, and *Cornus stolonifera* also occurred. Owing to utilization of the forest as pasture land, the shrubby growth was only poorly represented.

The mature soil profile is well developed, showing that it has been undisturbed by excessive erosion, except in very recent years. The soil is of glacial origin. There was only a small percentage (about 10) of particles coarser than fine sand, and the fine sand rarely exceeded 15 per cent. The bulk of the soil at all depths to 12 feet consisted of about equal proportions of very fine sand, silt, and clay, with the clay fraction preponderating at depths beyond 12 inches. Horizon A, or zone of extraction, from which the carbonates have been leached and much of the clay alluviated, extends to a depth of about 18-20 inches. It has an excellent granular structure and a water-retaining capacity (HILGARD method) of approximately 60 per cent. A thin layer of humus occurred only in the surface inch. Horizon B, or zone of concentration, where much clay has accumulated, is about 22 inches thick, reaching a depth of 40 inches. Much of the lime has been leached from this horizon. The shrinking of

the soil in drying and its cleavage into long perpendicular columns give the soil of this horizon a distinctly columnar structure. At greater depths, in horizon C, where neither extraction nor accumulation of materials has occurred as a result of soil-forming processes, the soil has a massive structure. This horizon extends far beyond the depths of the roots of the trees. The carbonate content is high,



FIG. 2.—Portions of main lateral roots of bur oak 40-55 feet in length. The stand of oaks in background is somewhat denser than where excavations were made.

streaks of chalky material being somewhat in evidence. The soil is friable, more easily penetrated by roots than is the columnar layer above, and prismatic cleavage becomes imperfectly developed. With increasing depth the color likewise changes. The very dark brown of horizon A intergrades into the grayish brown of the deeper one, and this in turn shows a transition to buff in the massive layer.

ROOT SYSTEM OF SEEDLINGS

In regard to the establishment of the individual tree, it has been shown (23) that the initial root habit is an important and often a

determining factor in the survival or death of the seedling. The amount of water and nutrients that will be made available to the seedling depends upon the initial root habit, which it seems is in turn dependent upon the species and is not in most cases at once modified by external conditions.

An adaptation of the bur oak to dry sites is shown in its extending the taproot deeply, usually about 9 inches, before unfolding the leaves above ground (12). Moreover, the root continues to penetrate the soil at a rapid rate and thus avoids succumbing to drought during summer when the surface layers may become dry. The senior writer has traced the development of the seedlings where the acorns were planted among the prairie grasses. A depth of more than 3 feet was attained the first year. More recently HOLCH (12) has studied the root activities of this species in cultivated soil on a cleared hilltop in eastern Nebraska. At the end of the first growing season a depth of 5 feet and a total lateral spread of 30 inches were attained. By the second autumn it had extended 2 feet deeper and the lateral spread had reached 38 inches. An examination of 3-year-old seedlings showed that the taproot had reached a depth of 8.5 feet and some of the main laterals had grown so vigorously that the total spread of branches was 5.2 feet. This rapid growth explains the ability of the bur oak to thrive on upper slopes and exposed hilltops in soil so dry that all other native trees fail. A study of its very abundant laterals which clothe the taproot from near the soil surface to the maximum depth of penetration also explains how this species can compete successfully for water with the shrubs and grasses.

The rapid growth of lateral branches, some near the soil surface, during the third year is of interest. In its younger stages this tree has, generally speaking, only feeding roots; later it develops anchorage roots.

EXCAVATION OF MATURE ROOT SYSTEM

A mature tree 65 years old, 37.5 feet tall and 14 inches in basal diameter, was selected for the initial excavation. A portion of the root system near the trunk was exposed as a result of erosion. This had occurred almost entirely within recent years and after the tree

was nearly mature. It was the result of close pasturing with both cattle and swine.

The general plan of procedure was first to excavate the taproot and those parts of the laterals that occurred within a radius of 12 feet from it. Afterward the branches extending beyond this area were traced. This method was followed in order to reduce, so far as possible, the great labor involved in removing a large volume of soil, the original excavation being filled in the process of obtaining the long lateral branches.

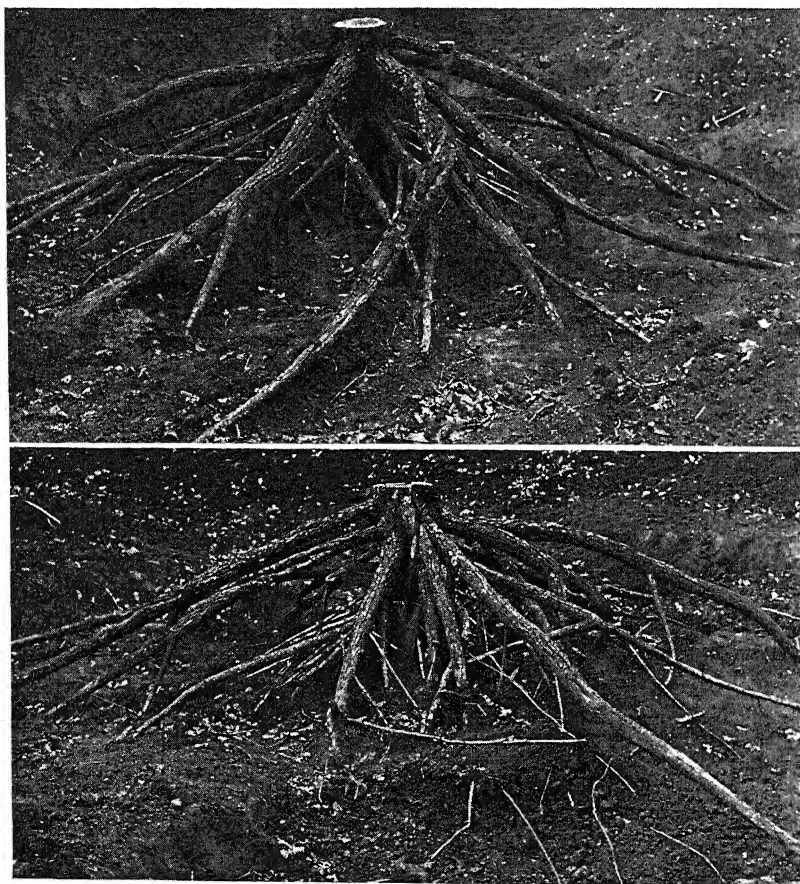
PRIMARY LATERALS.—All of the soil of horizon *A* was carefully removed from the circular area and most of the major roots exposed to a depth of about 2.5 feet (figs. 3, 4). Each primary lateral was numbered and measured, and the number, diameter, and direction of growth of its branches were recorded. The branches were then severed from the primary lateral and temporarily left in place. The main root was cut where it branched from the taproot and also at the place where it left the excavated area, or entered the deeper soil within it. Each root was marked by means of a numbered stake driven into the soil, so that it could be further examined after the taproot and its branches within the primary area of excavation had been studied.

There were 15 major branches, 2–7 inches in diameter, that had their origin on the first 15 inches of the taproot. Seven others, varying from 2 to nearly 4 inches in thickness, arose from the taproot between 15 and 36 inches. The depth of origin, diameter, length within the primary excavation, and diameter at the distal, severed ends are shown in table I.

Table I indicates the large size and widely spreading habit of the main roots. Root no. 12, for example, was 4.5 inches thick. It originated 8 inches below the soil surface, and ran outward a distance of 13 feet where it was still 1.2 inches thick but only 30 inches deep. All but four of the fifteen uppermost roots grew outward at so small an angle from the horizontal that they left the 12-foot excavation on all sides of the tree at an actual soil depth of only 15 to 32 inches. The other four extended more obliquely downward. Among those originating deeper, five of the seven behaved like the first eleven. It must be recalled, however, that the original depth was somewhat

greater, perhaps 16 inches, since the soil on this hillside had been eroded about this particular tree.

The root systems of two other trees, one 18 and one 14 inches in diameter, were excavated to determine whether or not the one de-



FIGS. 3, 4.—Fig. 3 (above), root system of bur oak as it appeared after removing about 2.5 feet of soil. Fig. 4 (below), same as figure 3 but from opposite side of tree.

scribed was unusually well furnished with large laterals and also to secure further information on their depth of origin, direction, and depth of growth. The data resulting from this study of the branches originating from the first 40 inches of the taproots are given in table II (cf. fig. 5).

An examination of table II shows that the three trees had 22, 27, and 26 main roots respectively over 2 inches in diameter; nine, seven, and seven 1 inch thick; and that the third tree was especially well supplied with branches of smaller size. The depth of origin of the large laterals was also very similar to that of the tree previously described, although only a little erosion had occurred. The bulk of the major laterals in all cases originated in the first 2 feet of soil;

TABLE I
DATA ON MAIN LATERALS TWO OR MORE INCHES IN DIAMETER

ROOT NUMBER	DEPTH OF ORIGIN ON TAPROOT (INCHES)	DIAMETER (INCHES)	LENGTH UNCOVERED (FEET)	DEPTH WHERE CUT (INCHES)	DIAMETER WHERE CUT (INCHES)
1.....	15	3.8	12.0	20	0.5
2.....	8	4.2	12.0	15	1.2
3.....	5	4.1	5.5	20	2.0
4.....	15	6.2	6.0	26	2.0
5.....	11	3.9	9.0	26	1.5
6.....	5	4.0	11.0	24	1.0
7.....	10	4.8	14.0	30	1.0
8.....	10	4.7	11.0	32	2.0
9.....	14	6.2	10.0	31	1.5
10.....	14	2.0	6.5	30	0.7
11.....	8	6.9	15.0	29	1.7
12.....	8	4.5	13.0	30	1.2
13.....	9	4.0	3.4	20	2.5
14.....	14	2.7	12.0	29	1.5
15.....	15	4.0	12.0	32	1.2
16.....	32	2.0	12.5	24	0.5
17.....	36	2.5	12.0	30	0.2
18.....	22	2.2	10.0	32	0.9
19.....	26	2.5	11.0	38	0.7
20.....	24	3.0	9.0	37	1.0
21.....	28	2.0	5.0	33	1.1
22.....	25	3.7	10.0	31	0.7

many occurred near the soil surface; and relatively few were found at depths greater than 2 feet. The characteristic direction of growth was also similar to that already described; most of the roots ran far outward, some horizontally, mostly with only a slightly downward course.

The major laterals nearly always pursued a direct course, often with long graceful curves, and with few exceptions no abrupt turns were encountered. Moreover the roots tapered gradually and, except where major branches arose, very uniformly. For example, root no. 2, with an original diameter of 4.2 inches, tapered to 3.5

inches at 6 feet, 2.5 inches at 9 feet, and was 1.2 inches thick where cut at 12 feet from its origin. No. 12 was 4.5 inches thick but had diameters of 4, 3.5, and 1.2 inches at 6, 8, and 13 feet respectively.

TABLE II

NUMBER OF MAIN LATERALS OF VARIOUS SIZES (IN INCHES) ORIGINATING ON THE FIRST 40 INCHES OF THE TAPROOTS

TREE	INCHES												
	DI-AMETER	0.25 OR LESS	0.25-0.5	0.5-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10
1.....	14	7	3	0	9	7	4	8	0	3	0	0	0
2.....	18	0	0	2	7	8	7	2	4	2	2	1	1
3*.....	14	4	5	16	7	8	8	9	0	0	1	0	0

* On this tree 25 of the laterals, nearly all large ones, originated from the first 22 inches of taproot.

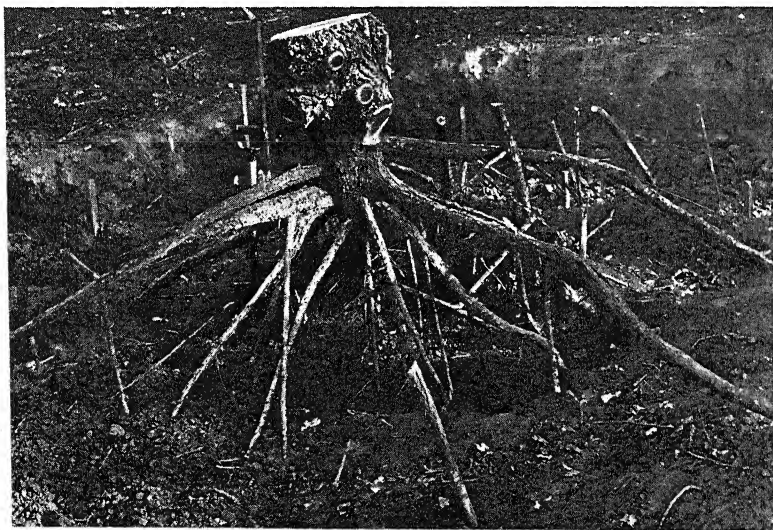


FIG. 5.—Taproot system after the large, upper lateral branches had been removed; note that the branches originating deeper take a more oblique course. Vertical roots are branches (sinker) from the main laterals. The stakes mark places where the main branches were cut.

Grafting of roots of the primary laterals, and in fact, of roots of smaller sizes, was very much in evidence on all of the trees examined.

SECONDARY LATERALS.—The number of branches of the second order that arose from the parts of the roots thus far unearthed was

great. Their distribution was irregular but most of the larger ones had their origin within 6 to 8 feet of the taproot. The numerous smaller ones in general arose from the more distal portions of the main roots. A total of 82 larger branches ranging in size from 0.25 to 4 inches were counted and measured. Twenty-nine belonged to the first group (0.25 inch), twenty-four were 0.5 inch thick; and the numbers of larger sizes, increasing in diameter by 0.5 inch respectively, were 9, 7, 3, 5, 2, 2, and 1. The direction pursued by these branches was variable. A few ran horizontally; many spread laterally, forming a 20° – 30° angle with the main root and then almost paralleled its course. Others ran obliquely outward and downward. These branched similarly to the main roots, which will later be more completely described. But fully one-fifth of the branches grew so nearly vertically downward that they may be characterized as "sinkers."

The branching of the sinkers, which varied in diameter from 0.2 inch or less to 2.5 inches, was characteristic and their downward extent rather great. They were clothed with widely spreading branches, both large and small, which rebranched repeatedly, resulting in a large absorbing area. They may best be understood by a description of a few typical examples (fig. 6).

One sinker originated from a main root 18 inches from the taproot and was one of five similar branches arising at various places in the first 4 feet of its course. Its original diameter was 2 inches. It penetrated almost vertically downward, tapering gradually and rather uniformly to a depth of 7.5 feet where it was only 5 mm. thick. Thereafter the diameter was almost uniform to a depth of 15 feet. A large branch, 0.75 inch in diameter, arose at 12 inches from the proximal end. It ran obliquely downward at an angle of 45° . This branch gave rise to many laterals, for example five 0.25 inch in diameter and twelve 1 mm. thick, in the first 3 feet of its course. A lateral 5 mm. in diameter arose 6 inches above the large one described, and another 0.7 inch thick arose at about the same level. There was a total of thirty branches in the next 5 feet. They arose at irregular intervals, and varied from 1 to 10 mm. in diameter. Nearly all ran outward at angles of 15° to 45° from the horizontal but a few penetrated more vertically downward. They spread widely and branched

profusely. Two laterals, 3 and 5 mm. thick respectively, arose at 7.5 feet depth. The larger one ran obliquely for a short distance and then turned downward and more or less paralleled the course of the main root, the size of which it approximated. Below 7.5 feet the branches were small and rather numerous but short. Only a few threadlike laterals occurred below 10 feet, and just beyond a depth of 15 feet the root ended abruptly in the hard, dry soil.

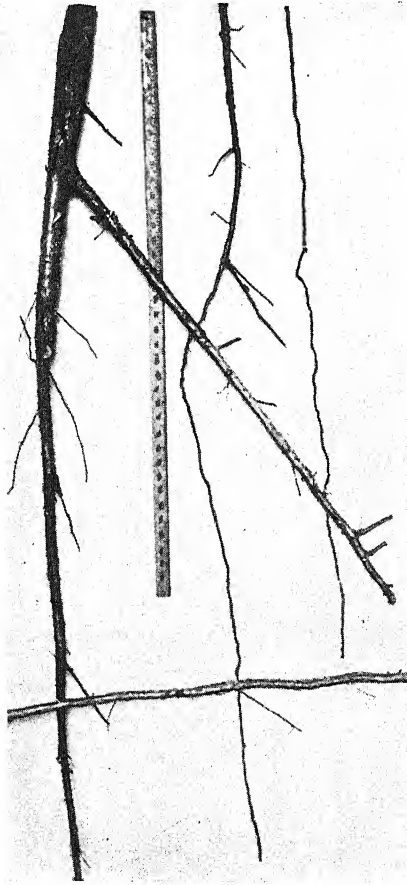


FIG. 6.—Sinkers about 15 feet long in three vertical sections; note rather numerous branches. Running horizontally across the bottom is a portion of one of the numerous ropelike roots.

Another sinker, originating from a major lateral 4 feet from the taproot, was 2 inches in diameter. Like the preceding, it penetrated vertically downward and tapered gradually. Branches of various sizes arose in profusion. Fourteen major branches, 5 to 15 mm. in diameter, and an equal number of smaller ones occurred in the first 4 feet of its course. These had threadlike sublaterals in abundance, all of which were clothed with masses of absorbing rootlets. The direction of growth was similar to that already described. At greater depths the

branches became more threadlike and fewer in the harder soil. Maximum penetration was about 15 feet.

Still another sinker gave rise to seven major branches (0.25–0.5 inch thick) and eight smaller ones in the first foot of its course, and

thirty-five in the next 4 feet. It then penetrated to the 10-foot level, branching more sparingly. Since several of the larger branches ran obliquely outward for distances of 10 to 15 feet and all branched freely, the total soil mass penetrated by a single sinker is very great.

While a few of the sinkers examined had no large laterals and relatively few smaller ones, the preceding descriptions are typical of the group. The individual sinker develops in a manner very similar to that of a sapling oak, and if its origin were unknown it might readily be confused with a small taproot system.

TAPROOT.—The taproot was 14 inches in diameter at its origin, that is, near the old soil line and where the large lateral roots originated. But it tapered so rapidly, after giving rise to the numerous large branches, that at a depth of 3.3 feet it was only 4.5 inches thick. Its width decreased to 3.7 inches at 5 feet in depth and to 1.75 inches at the 6-foot level, after giving off a branch equaling its own diameter (fig. 7). In addition to the twenty-two roots in table I, sixteen others occurred on the first 3 feet of the taproot. Ten of these were of large diameter (0.25–1.6 inches); the others were only 2–3 mm. thick. Nearly all penetrated much more obliquely downward than the roots already described. Between the depths of 3 and 7 feet, laterals were much fewer and of smaller size; in fact there were only two with a diameter greater than 1.5 inches. Seven branches were .4–1.5 inches thick; fourteen others were 0.2–1 inch in diameter; and three were of smaller size. This made a total of sixty-four primary branches from the first 7 feet of taproot, of which 60 per cent (including nearly all the larger ones) originated in the first 3 feet.

From the 6-foot level the taproot (now only 1.75 inches thick) branched sparingly and pursued a tortuous downward course. Three branches (included above) arose in the seventh foot. These were 0.75, 0.5, and 0.3 inch thick. Six other small laterals (4–5 mm. thick) were also found. The taproot was now reduced to a diameter of only 0.75 inch. In the eighth foot a single horizontal lateral 0.3 inch thick arose. At 9 feet in depth the main root gave rise to a small branch 4 mm. in width which ran outward and upward. The taproot, of similar small dimension, pursued a tortuous downward course through the hard soil. Another branching reduced its size to 2.5 mm.

and still another to 1 mm. It was now at about the 10-foot level. Only a few other threadlike branches were found at this depth. The brown, brittle, threadlike taproot and several of its branches pur-

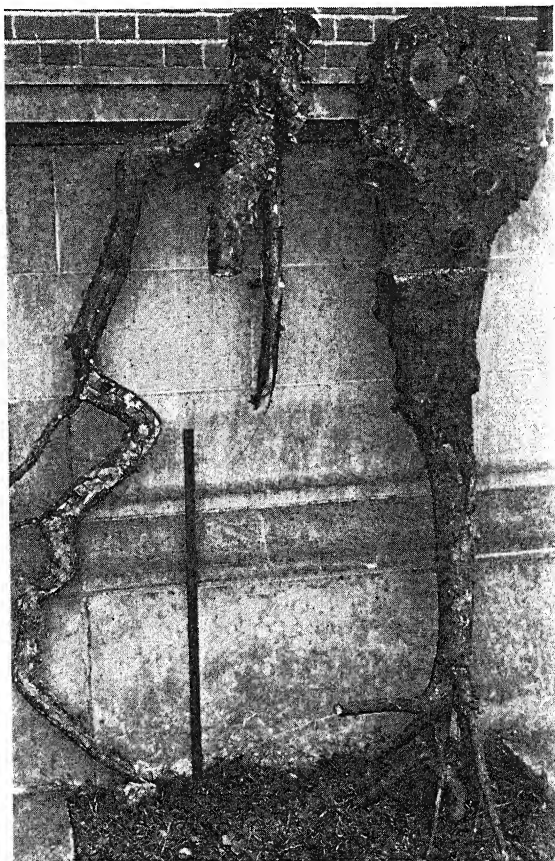


FIG. 7.—Portions of two taproots: the one on right is from tree no. 1 and is about 6 feet long; the other, cut at depth of the chalk line on the first, is from tree no. 3 which maintained a larger diameter (note crooked shape).

sued parallel downward courses, branching sparingly. Many of the branches were decayed near their ends and none, including the taproot, penetrated beyond 14 feet in depth.

The rapid tapering of the taproot and its lack of greater penetration initiated further study. Oaks no. 2 and no. 3 were felled and

their taproots examined. That of no. 2 tapered from a diameter of 18 to 5.5 inches in the first 3.3 feet of its course. A foot deeper it was only 1.5 inches thick and at the 5.5-foot level, 0.5 inch. Thus the taproot was again poorly developed.

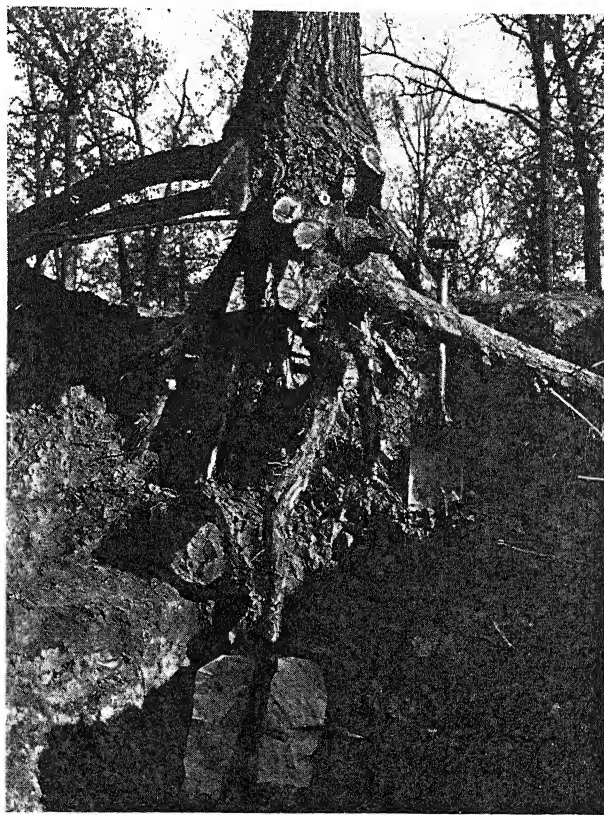


FIG. 8.—Root system of tree no. 3; taproot shown to depth of about 6 feet

On the third oak the main root lost half of its original thickness (14 inches) in the first 2 feet. At a depth of 5 feet it was only 2.7 inches thick, but at the end of the twelfth foot, the root having been much flattened and distorted for several feet, the diameters were 1 and 2 inches respectively (fig. 8). Here it ran horizontally for some distance, gradually narrowing and finding great difficulty in penetrating the hard soil. It probably penetrated somewhat deeper than

the first taproot examined. It should be noted also that the portion of this taproot between the 3.3- and 12-foot levels was much more poorly branched than the first one described. In fact, only thirteen branches were found on this part. All but four were less than 0.5 inch in diameter.

Several other trees, where the taproots had been partly exposed by erosion, yielded similar data. A much larger specimen with a diameter of 3.3 feet was studied on a high, level terrace which the Weeping Water River had eroded. The taproot system was fully exposed for a depth of 9 feet. It was much smaller than a score of the main lateral roots and measured only 3.5 inches in diameter at 4.5 feet depth. The diameter, however, was nearly as great at the 9-foot level.

From these studies it may be concluded that the taproot of the bur oak, at least in the type of soil where it was studied, is less extensive than many of its major branches.

ROOT DISTRIBUTION NEAR THE TREE; ROOT TYPES.—A clear description of the network of roots occupying the soil within the 12-foot radius of the tree is somewhat difficult. In addition to sixty-four main branches of the taproot, which occurred primarily in the first 2 to 4 feet of soil, there were eighty-two large secondary roots (0.25-4 inches in diameter). About one-fifth of these were sinkers, each often having a root system like that of a sapling oak. In addition, many roots originated from the more vertically descending main laterals which were not uncovered (and hence not included) in the earlier excavation. These, with branches, both large and small, from their secondary laterals, and also the very numerous branches of secondary roots that were originally uncovered, formed an intricate root pattern.

The area within a radius of 10 feet of the tree (except close to the taproot) was especially well occupied by roots of various sizes. Aside from the large, thick roots already described, three other types predominated, and indeed intermediate sizes were relatively scarce. First there were the large ropelike roots, usually 0.5 inch or somewhat less in diameter, which ran long distances (5-20 or more feet) with little decrease in thickness (fig. 6). They and the larger branches, including the main laterals, gave rise to an abundance of

tough, reddish brown, cordlike roots that were usually 3-5 mm. thick, and maintained a uniform diameter for distances of 5 to more than 10 feet. These ran in all directions, often even vertically upward or downward, and threaded the soil with an efficient network which, in part, was the source of the third general type, that is, the capillary branches. While these cordlike roots sometimes ran 2 feet or more with scarcely a single lateral, more usually there were five to eight branches per foot and sometimes as many as twenty to twenty-four.

These cordlike roots (and even finer branches) arose from the larger roots and, in fact, also from the main laterals. Sometimes they were sparse but often abundant. Frequently as many as twelve to twenty per foot were counted; sometimes there were several feet of main roots with none.

In general the first 6 feet of soil was filled with a well developed root system, approaching in places such a network as is often found among herbaceous plants (26, 29). The root network decreased rapidly at depths of 6 to 10 feet, and at greater depths roots were sparse.

The smaller laterals, which undoubtedly carried on the major part of the absorption, warrant careful consideration. In fact, the description thus far has dealt with the skeletal framework from which the absorbing roots arose. This is true even where the non-absorbing roots form a tangle below ground (as was found within 10 feet of the tree) similar to a thicket of branched shrubbery and small trees above the soil. Figure 9 shows a few smaller roots and the abundance of much branched rootlets.

Many of these fine rootlets, especially in the surface soil, come from obliquely or vertically ascending branches which often originate several feet in depth. For example, one root 0.4 inch in diameter was cut from a main horizontal lateral. It ran vertically upward 28 inches, gave rise to several large branches, and then turned nearly horizontally in such a manner that many of the ends of the profuse ultimate laterals came within 0.25 inch of the soil surface. Eleven branches 1 to 4 mm. in diameter had their origin in the surface foot of soil. They spread widely, branched repeatedly, and their mats of ultimate rootlets almost filled the rich surface substratum.

Many branches with absorbing rootlets were floated out in water and examined in detail. The following examples are representative of well branched roots. A section of a root 9 inches long gave rise to eight branches 1 mm. thick and forty-two of smaller size. The larger

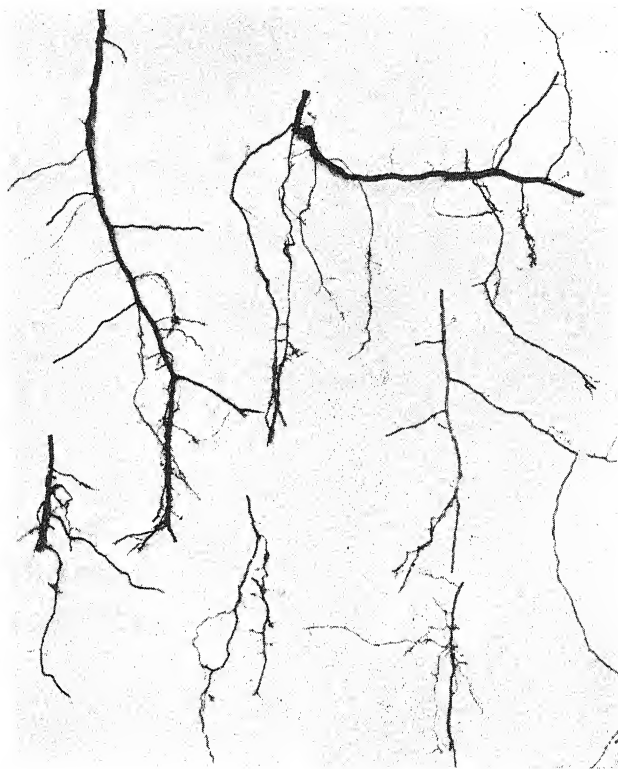


FIG. 9.—Selected fragments of roots showing usual degree of branching

ones rebranched at the rate of twelve rootlets per inch; the finer ones had seventeen to twenty-five fine laterals per unit length. Many of these were rebranched three or four times. Another root section 5 inches long had seven millimeter branches and twenty-eight threadlike ones. Branching at the rate of fourteen laterals per inch was common on fine rootlets, and five or six per inch occurred on those that were only poorly furnished with laterals.

The root termini commonly had as many as twenty branches per inch, mostly 0.5 inch or less in length but so completely rebranched that the soil was extremely well filled. Mycorrhizal mats, yellow, white, or pink in color, were found in great abundance. Here the root mass was often flattened into one plane, forming densely appressed mats or "fans" 0.5–1 inch wide. Often fifty or more short branches per inch occurred in such areas (fig. 10). The mats were especially well developed throughout the first foot of soil but also occurred deeper. The whole horizon *A* within a radius of 12 feet from the tree was well threaded with the absorbing rootlets. As the roots extended many feet farther, repeated divergence of branches continued to afford a skeletal network for the origin of more fine laterals; but here, of course, the whole territory was not so fully occupied by the roots of an individual tree.

As regards color, all of the roots whether large or small were some shade of brown, varying from grayish to reddish brown. They possessed great tensile strength, being characteristically as "tough as oak."

Upon the death and decay of the roots, channels are left in the soil. One channel 0.5 inch in diameter was traced at the 10-foot level for a distance of more than 4 feet. Many smaller ones were found. Some were partially lined with fragments of old bark; others contained a new growth of roots. Undoubtedly the larger channels, at least, remain in place for long periods of time. Dead root ends are often found on vigorous forest trees, and sometimes entire roots or at least considerable portions of them may die and decay.



FIG. 10.—Ultimate absorbing laterals showing also some mycorrhizal "fans."

EXTENT AND BRANCHING HABIT OF PRIMARY LATERALS.—As regards root extent beyond the primary excavation, the following examples are illustrative. Root no. 8 was cut 11 feet from its origin where the diameter was 2 inches. Within a foot it forked dichotomously. One branch ran obliquely downward; the other ran outward almost horizontally and at a depth of about 2 feet. The latter immediately gave rise to a branch that also ran horizontally, making an angle of 25° with the main root. The main root tapered to a diameter of 1 inch 3.5 feet farther out, and after giving rise to two branches each 0.25 inch thick it was only 0.5 inch in width. One of these branches ran vertically downward; the other horizontally outward. Throughout its entire course this root gave rise to many small branch roots 3 to 5 mm. thick. These extended in all directions, as frequently upward as laterally or downward. At a distance of 30 feet from the taproot, and where the main root was only 4 mm. thick, it turned obliquely downward. The main root now divided into numerous smaller rootlets which extended outward and downward approximately 8 feet farther.

The other main branch of no. 8 soon pursued a course more or less parallel with the surface soil at a depth between 2 and 3 feet. It also extended outward (31 feet) before running obliquely downward. Its branching was similar to that of the other fork. The tip was only 3 mm. in diameter and was not traced beyond 5 feet in depth.

A branch of no. 8, 1 inch thick, which arose at a distance of 10.8 feet from the trunk, also ran far outward. It soon branched dichotomously, one branch running downward at an angle of 20° from the horizontal. The other branch was traced outward in the second and third foot of soil 23 feet farther. Here it divided into several branches only 3–5 mm. thick. These branched again and again, running outward and downward about 10 feet more. The root was ropelike and tapered so slowly that for many feet change in diameter was scarcely perceptible. Few large branches were found and smaller ones originated only intermittently. Only six laterals of various sizes arose in the first 5 feet of its course, but on other parts there were twelve to eighteen small branches per foot.

This main lateral with its widely spreading major and minor

branches, all covered more or less profusely with absorbing rootlets, well illustrates a small part of this really wonderful anchoring and absorbing system. Sometimes a root turned upward a foot or more, or abruptly downward, but the curves were nearly always remarkably gradual and graceful compared with those of many other species of plants. The direction of growth of even isolated roots could easily be determined by their branching habit. Roots of all sizes, including the taproots and main laterals, showed a decided tendency to give rise to one or more branches where they did bend abruptly.

Often the main roots enlarged 0.5 inch or more in diameter if the soil was eroded from them or if in their course they appeared at the surface. This was due, in part, to the increase in the development of the thickness of the bark.

Further description of the lateral roots seems unnecessary; in fact, all of them were not examined. Instead, the roots of certain other oaks on gently sloping ground were studied since they were somewhat easier of access. Only two will be described.

The first was 3.6 inches thick at its origin which was 12 inches deep. It ran outward at a rather uniform depth between 2 and 2.5 feet. Thirty feet outward it was still 1 inch thick. It was traced 10 feet farther where it was 5 feet below the soil surface. Here it turned abruptly downward, the diameter increasing to 1.5 inches. It tapered rapidly in the next 3 feet of soil but was not traced to its end. Five large branches (0.25–0.75 inch thick) and very numerous smaller ones arose beyond the 12-foot radius and two between 30 and 40 feet. In addition, smaller laterals were abundant.

A main lateral was examined on another tree that had a trunk diameter of 14 inches. It was 3 inches thick and originated at a depth of 2 feet. This root pursued a gently curving, nearly horizontal course at a depth of about 28 inches. The diameter decreased at 10-foot intervals to 2.75, 2.5, 1.75, 1.25, and 1.0 inches respectively. At 54 feet from the tree it turned directly downward, and although this part was unearthed only a few feet, it probably penetrated deeply. Three major branches 0.5 to more than 1 inch in diameter arose in each of the three distal 10-foot sections. They ran either horizontally or obliquely upward or downward and rebranched in a manner not unlike that of the main root. Almost countless numbers

of small rootlets originate from such a major root and its network of laterals, and the total volume of soil laid tribute by a single major branch is really enormous.

Other main branches were traced for distances of 40 to 56 feet and one to more than 60 feet. This was two to four times greater than the radius of the crown of the tree (fig. 2).

RELATIVE WEIGHT AND VOLUME OF TOPS AND ROOTS

The tree was cut at the original ground line and the top divided into convenient sections for weighing. Although the leaves were drying, few had fallen and their weight was included with that of the trunk and branches. The total weight of the top was 1285 pounds.

The large main roots from the upper portion of the taproot, except a few of the ends, were collected and weighed. The weight of the termini (which was not great) was approximated from the weight of similar roots of average length and equal diameters. All of the major roots, over 5 mm. in diameter, from one-fourth of the area penetrated by the root system below 3 feet, were also collected and weighed and the weight quadrupled. These weights, with that of the taproot itself, gave a total which was just equal to the weight of the tops. Since the soil was dry, the wood of the roots probably contained little more water than the branches. Thus this specimen had a skeletal root system which equaled in weight that of the parts above ground. The significance of this from the standpoint of absorption is not great. It merely indicates the enormous development of the anchorage system and at the same time the extent of the framework upon which the absorbing, fibrous network of roots develops. Had all of these fine roots been collected as well, their weight would have added little to the total, but would probably have caused the weight of the roots to exceed that of the tops.

The relative volumes of roots and tops were also approximated by determining first the weight and then the volume of average samples of roots as well as tops, each of three different sizes. Since the weights of roots and tops were approximately equal, the volume of the roots, because of their greater density, was about one-tenth less than that of the wood above ground.

Discussion

One of the most striking features of the root system of the bur oak is its wide lateral spread. BÜSGEN and MÜNCH (6) state: "At the margins of woods, tree roots are not seldom to be met with at a distance of 20 meters in the adjoining fields. For this reason cultivated ground near woods must be protected from the roots of trees by means of trenches."

PEREN (20) studied the roots of fruit trees in relation to spacing in the orchard as well as the length of time "filler trees" or bush fruits should be allowed to remain without seriously competing with the permanent trees. A 15-year-old Norwegian cherry had a lateral spread of roots of 30 feet. The roots of certain varieties of 16-year-old apple trees extended to a maximum distance of 24.3 feet from the base of the tree.

GEMMER (9) has shown that a long-leaved pine, *Pinus palustris*, 3 inches in diameter and 20 feet tall, had extended its roots 8 feet laterally through the sandy soil. He states that on mature trees roots 0.5 inch in diameter are not infrequently found 30 feet from the tree, a distance twice the radius of the crown.

LAITAKARI (14) found that the longest roots of *Pinus sylvestris* occur on sandy heaths. The greatest lengths determined were 17-25.5 meters. These findings are similar to those of ALLAN (4), who states that the roots of a matai (*Podocarpus spicata*) 15 meters tall, in a rain forest of New Zealand, had a lateral spread of 19.5 meters from the base of the tree.

There is considerable evidence that the roots of trees developing in poorer, lighter, well aerated soils tend to grow longer and often thinner and straighter than those in more compact and richer soils which are shorter and often thicker, pursuing a more devious course. Since the bur oak grew in fertile, compact, but well aerated silt loam soil, its great lateral extent cannot be attributed to these factors of the substratum. It would seem that the scarcity of the water supply, at least during recurrent periods of drought, is the chief external factor promoting extensive root development. This has repeatedly been determined experimentally for various herbaceous plants, and also undoubtedly holds for trees (13).

LAITAKARI (14) finds that the size of the tree and the length of the root system are rather closely dependent upon each other. It thus seems clear that the small size of the trees of these marginal oak forests is limited by the ability of the root system to furnish sufficient water for greater growth. An open stand where the crowns do not touch is indeed a closed one so far as the overlapping root systems and the supply of the dominant limiting factor, water, are concerned.

The more or less vertical growth of roots from the deeper, large horizontal or oblique branches is a rather common phenomenon among trees. For example, it has been found in Europe (HILF 11) that the roots of the beech do not extend beyond the crown but run downward more or less diagonally to various depths. Branches from these deep roots extend upward to the humus layer, where they re-branch profusely in the richer surface layers of soil. In fact, HILF states that notwithstanding the deep skeletal root system, the beech is especially dependent upon the humus layer for its nutrients. PELHAM (19) found that the large primary laterals of the pecan extended widely at depths between 2 and 5 feet. They gave rise to an abundance of vertical "feeding roots" 0.25 inch or less in thickness which extended nearly to the surface of the soil.

The intergrowth of roots is a common phenomenon in many forest trees. It is found most abundantly within a few feet from the base of the tree. Both horizontal and vertical roots are concerned; in fact the two types were frequently grafted one into the other. LAITAKARI (14) states that in the Scotch pine, roots are frequently found which only connect two other roots and do not grow any farther. He suggests that the main purpose of this kind of "bridge-root" seems to be to strengthen the root system. None of these were seen in the oak.

The phenomenon of root intergrowth is easily understood when the very firm structure of the soil is considered. The soils of horizons B and C were both very hard and difficult to remove, even with a hand-pick. The extreme compactness of the soil about the roots, increasing with root diameter, although not surprising is remarkable. As a result of growth, the taproot and its large branches compressed the soil about them so greatly that it was removed only with much

difficulty. Two young roots coming into contact would exert in their enlargement an enormous pressure, not only against the soil but also against each other. Under such conditions grafting would be almost an inevitable result.

One very noticeable phenomenon observed in the excavation was the small effect a first or second generation of trees had upon the prairie soil. In the columnar layer there was, as in the case of herbaceous plants of the prairie, a marked tendency of the roots to follow the cleavage planes between the columns and form a network of absorbing rootlets which spread out over their surfaces. BURGER (5) has shown the important part played by the roots of trees in preparing suitable soil for forest growth. Numerous investigators have found that the roots of trees frequently grow along channels of decaying or decayed roots of former generations. Here they meet little mechanical resistance, have an abundant supply of air, usually also plenty of water, and immediate access to the nutrients as rapidly as they are liberated in the process of decay. In some cases they seem to be of importance in directing the roots to the ground water. Coniferous roots develop especially well where the roots of hardwoods have made deep root channels.

That the adaptation of a species to its habitat is largely a matter of root development is a viewpoint that is being strongly supported by rapidly accumulating evidence. Open stands in forests are caused, not so much by intolerance to shade perhaps as by competition of widely spreading roots. AALTONEN (1) has reached the conclusion "that the space arrangement of those parts of the trees which are above the soil is mainly decided by their roots and the competition existing between them for the water and food in the ground." Other investigators have been led to a similar conclusion. BÜSGEN and MÜNCH (6) point out that the number, direction, and size of root branches express first of all adaptations to the environment, and that broad-crowned, deeply branched, isolated trees have therefore much larger masses of roots than those confined in dense woods. ADAMS (2) has shown that close spacing results in a marked change in the root form of jack pine, modifying its widely spreading root system into a vertical form with much branched, short, stubby roots.

The severest competition between individual trees takes place in

pure stands, such as the bur oak, since the roots tend not only to occupy the same layers of soil but to develop at about the same time. In mixed stands such as pine, birch, and spruce, competition among the roots has been shown to be much less severe than in an equally dense stand of a single species. This is due to the fact that the horizontal roots of these species occupy the soil at different levels. Thus in Europe an undergrowth of birch often thrives under spruce (15). Some interesting cases have been found where one species of tree has actually destroyed another by root competition, although the crowns did not touch. *Populus canadensis* has thus been replaced by *Elaeagnus angustifolia* and *Machura aurantiaca* by *Ulmus campestris* (17).

From these data we may conclude that the small size, usually wide spacing, and very extensive root system of the bur oak are adaptations to a relatively dry soil and atmospheric conditions scarcely congenial to tree growth. Its successful competition against the grasses and shrubs is made possible by the rapid growth and deep penetration of the young root system and by its widely spreading habit which enormously increases the soil volume from which water may be secured to meet the needs of the growing top.

Summary

1. An ecotone between the grassland and the deciduous forest along the Missouri River occurs in southeastern Nebraska. The mean annual precipitation is 28-32 inches; humidity is relatively low, evaporation and wind movement are relatively high; the silt loam soils are deep but during drought contain only a small amount of water available for growth. Thus competition for water between the grasses and the invading shrubs and trees is great.

2. *Quercus macrocarpa* is the most xeric forest tree. Since the cessation of prairie fires it is invading the grassland either directly or in the wake of the *Rhus-Symphoricarpos-Corylus* chaparral.

3. The shrubs advance largely by means of rhizomes into the grassland. Their roots often extend outward and then upward under the grasses. Those of *Symphoricarpos* especially are so finely branched and abundant as to compete successfully with the excellently developed and extensive root systems of the plants of the prairie.

4. The oak seedling develops a deep taproot before the leaves are unfolded. The strong, finely branched taproot extends into moist soil 3-5 feet the first summer.

5. Mature trees 50-65 years old were 35-40 feet tall. 12-18 inches in basal diameter, and grew 10-40 feet apart in a pure stand.

6. The taproot gave rise to thirty or more large main branches, most of which arose in the first 2 feet of soil. It tapered rapidly and extended to a depth of 14 feet.

7. Most of the main branches, which varied from 1 to 7 inches in diameter, extended widely (20-60 feet) before turning downward. Some grew even deeper than the taproot. All branched repeatedly, and together they occupied a very large volume of soil.

8. Many branches of the main roots grew vertically downward 8-15 feet, each more or less resembling the taproot system of an oak sapling. Others extended obliquely or vertically upward and filled the surface soil with a mat of absorbing rootlets.

9. Ropelike roots, 0.5 inch or less in diameter, extended many feet without much change in thickness. A cordlike type, 3-5 mm. thick, was also abundant. A third type consisting of fine, much branched rootlets clothed the widely extending skeletal framework and furnished the bulk of the absorbing surface. Mycorrhizal mats were abundant.

10. The weight of the roots equaled that of the tops; the volume of the roots was about one-tenth less than that of the parts above ground.

11. Low water content of soil is compensated by a widely spreading, well branched root system. This may account for the wide spacing of the oak trees and the open forest canopy.

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A CYTOLOGICAL STUDY OF TULASNELLA

DONALD P. ROGERS

(WITH SEVENTY-NINE FIGURES)

Introduction

The fruiting layer of *Tulasnella* is a delicate bloom or film, waxy or mucilaginous, occurring most commonly on the lower surface of dead branches or logs or on the bodies of other fungi growing there. The fructification consists of a thin, very loose mat of hyphae, upon which the basidia are seated; the whole bears a marked resemblance to a tenuous *Corticium* or *Sebacina*. The basidium arises by the swelling of the terminal cell of a hyphal branch into a broadly pyriform body; from its summit are produced four oval sacs, each of which puts out from its apex a filament, and upon this bears a spore. The interest that the genus possesses lies in the organization of the basidium, the precise homologies of whose parts have not been conclusively demonstrated.

History

The TULASNES (27) first called attention to the peculiarities of the *Tulasnella* basidium. In 1872 they published a brief notice and a few figures which set forth its characters very clearly. Its points of resemblance to the basidia of both *Sebacina* and *Corticium* were so striking that these investigators noted it as an intermediate form and because of it withdrew an earlier statement that those two genera should be separated widely. They took their fungus to be *Corticium incarnatum* Fr. and allowed it to remain under that name.

In 1888, SCHROETER (23) published the description of his genus *Tulasnella*, "with basidia and spores formed in the same manner as in the fungus described by TULASNE." He agreed with the statement in the earlier paper concerning the intermediate position of the type. His new genus he listed as an "Anhang" to the Tremellinei, with the note that its inclusion within that group must remain doubtful. Later in the same year appeared PATOUILLARD's account (21) of his genus *Prototremella*. He held that the similarity between

the basidium of this fungus and that of *Corticium* was only superficial. In *Prototremella* the basidial appendage, rather than the basal cell as in *Corticium*, is the "organ essential to the production of the spore"; and in *Prototremella* the spore germinates to form a secondary spore borne on a promycelium. This functioning of the parts of the true morphological basidium as separate basidia, in the older biological sense, and this germination of the spore by renovation, are the criteria of PATOUILLARD's *Heterobasidii*, and so he placed his genus in that group, near *Tremella*.

In 1889, OLSEN (4) published his genus *Pachysterigma*, like *Prototremella* a synonym of SCHROETER's name. The genus was placed lowest in BREFELD's newly erected *Tomentellei*. Like the forms associated with it there, it was considered very primitive, since its basidia are not arranged in a regular hymenium. The basidium itself was regarded as an autobasidium.

The detailed cytological investigations upon two species of *Tulasnella* which JUEL (16) published in 1897 are not invalidated by his having described as a poroid tulasnellaceous form what was in reality a *Tulasnella* growing upon a *Poria* (17). He found that in *Tulasnella* the binucleate condition of the young basidium, karyogamy, and meiosis occur according to the scheme generally characteristic for basidia. In his *Muciporus* (that is, *Tulasnella*) *deliquescent* the first postmeiotic division takes place in the discharged spore, but in *T. thelephorea* it takes place in the basidial appendage. According to JUEL's conception the latter body is the actual basidiospore, and the cell cut off and freed from its apex is a secondary spore or conidium. By the possession of sessile spores germinating in place, JUEL characterized his family Tulasnellaceae. He placed it, as a transitional group, between the Dacryomycetes (to him autobasidial) and the Hymenomycetes.

VON HÖHNEL and LITSCHAUER (15) in 1908 rejected JUEL's interpretation and his family, and included *Tulasnella* among the Corticieae, "since its close relationship is unquestionable," and since "between the oval sterigmata of some species of *Tulasnella* and the subulate ones of *Corticium* all transitions occur." Likewise RAUNKIAER (22) rejected JUEL's conclusions. He found his justification rather in direct criticism of JUEL's arguments and observations.

Especially he questioned that any evidence had been presented to show that the inflated bodies upon the *Tulasnella* basal cell were in any degree separate entities. In the absence of such evidence he considered them not spores but only sterigmata, and the fungi in which they occur corticiaceous.

NEUHOFF, in his study of the heterobasidiomycetes (20), placed *Tulasnella* among them. According to his conception, the heterobasidium arises as a probasidium and is typically composed of two parts, a basal hypobasidium and one or more epibasidia which bear the sterigmata and spores. Thus in *Tulasnella* the pyriform basal cell (the hypobasidium) and its four appendages (epibasidia) together make up the basidium; the very slender filaments borne at the tips of the epibasidia are sterigmata; the spores upon them are the basidiospores. NEUHOFF placed *Tulasnella* in the line by which he held the Tremellaceae to have evolved from corticiaceous forms.

BOURDOT and GALZIN (2), like VON HÖHNEL and LITSCHAUER, noted that several species of *Corticium* show evidence of close relation to *Tulasnella*. Following PATOUILLARD, however, they retained the latter genus among the *Heterobasidii*.

Observations

Living material of five species of *Tulasnella* was available for the present study. *T. eichleriana* Bres., *T. fuscoviolacea* Bres., and *T. violacea* (Quél.) Bourd. and Galz. were collected near Iowa City a number of times during the winter and early spring of 1930-31 and again in the late autumn of 1931. *T. tulasnei* (Pat.) Juel was collected at two places near Iowa City in November and December of 1931. *T. lactea* Bourd. and Galz. was collected once, at West Okoboji, Iowa, in July, 1931. Material of *T. lactea* was fixed in Allen's modified Bouin's fluid and of the other species in the weaker Flemming's fluid. All species were sectioned at 5 μ , and *T. tulasnei* at 8 μ also. Slides of all species were stained in Haidenhain's haematoxylin and counterstained with phloxine or erythrosin; in addition, slides of *T. violacea* were stained in safranin, gentian violet, and orange G. Crush preparations of the first four species were used also; such preparations were first treated with a dilute solution of potassium hydroxide and then stained with phloxine. A portion of the type

collection of *T. anceps* Bres. and Syd. was available, in SYDOW'S *Mycotheca germanica*; crush preparations and free-hand sections of it were studied.

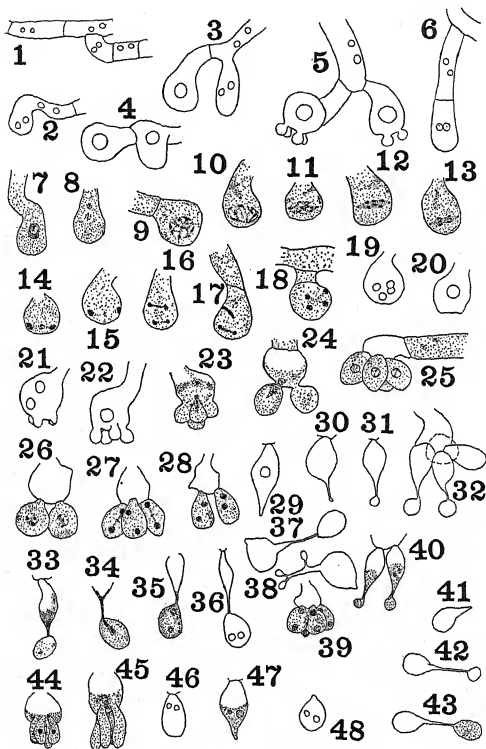
Material of *T. violea* appeared most favorable for cytological examination, and consequently received the most complete study. No more detailed account of the other species is given than seems necessary to supplement the observations upon the one species here regarded as typical.

TULASNELLA VIOLEA

The mycelium of *Tulasnella violea* penetrates the substratum to no great depth; occasional threads reach as deep as $300\ \mu$, but the greater amount of mycelium lies within $50\ \mu$ of the surface. The superficial layer likewise is very thin; most commonly in the material examined it measures only $20\text{--}30\ \mu$ to the summits of the basidia, although in some areas it is as thick as $50\ \mu$. The hyphae are usually $2\text{--}3\ \mu$ thick, and occasionally $4\text{--}5\ \mu$; septa occur at frequent intervals; the diameter of the cells may be uniform or may decrease from the middle to the septa. The mycelium appears to be entirely binucleate (fig. 1).

The majority of the basidia arise directly from the horizontal hyphae of the receptacle rather than from vertical basidiophores. Most commonly the terminal cell of a superficial thread turns outward, so that its tip is perpendicular to the substratum (fig. 2); the tip, separated by a septum from the horizontal portion, becomes a probasidium. Somewhat less commonly the long axis of the basidium lies parallel to the substratum (figs. 4, 25), and the basidial appendages appear on the outer side rather than from the apex. Occasionally basidia are borne on simple vertical basidiophores (fig. 5). Subterminal basidia also occur; the penultimate cell itself may swell into a basidium (fig. 4); or the basidium may develop as a proliferation of some cell of the receptacle (figs. 3, 18). The diameter of the youngest cells recognizable as basidia is no greater than that of the hyphae that produce them.

The two primary basidial nuclei come to lie side by side in the developing cell. Here they remain without visible change until the probasidium has attained about half its maximum diameter. Just



FIGS. 1-48.*—FIGS. 1-38, *Tulasnella violea*: Fig. 1, hypha of receptacle. Fig. 2, end of horizontal hypha, the out-turned tip a very young probasidium; basidial dikaryon formed and cell swollen, but probasidium not yet cut off by septum. Fig. 3, terminal

* All figures were drawn with the aid of camera lucida, using Zeiss objective 90X and ocular 15X, except figures 69-72 and 74-79, drawn under 90X objective and 10X ocular, and figure 52, drawn without camera lucida. As reproduced, figures 69-72 and 74-79 are X992, figure 52 about X1300, and all others X1390.

before fusion these nuclei increase somewhat in diameter (fig. 8); the fusion nucleus has about three times the original diameter of the primary nucleus. For a time after fusion the nucleus gives no evidence of further development; during this period the basidium increases considerably in size and becomes broadly pyriform (fig. 3).

Before division the nucleus is resolved into a spireme, considerably larger than the resting zygote, showing rather broad, deeply staining chromatin threads in a hyaline matrix (fig. 9). Presently this spireme gives evidence of polarity, becoming a broadly spindle-shaped structure reaching completely across the basidial cell near the summit (figs. 10, 11). From this is formed the much more slender mitotic spindle (figs. 12, 13). On the spindle a number of minute dark bodies—frequently six but sometimes, apparently,

basidium formed and nuclear fusion in it completed; younger probasidium in process of formation on penultimate cell. Fig. 4, terminal probasidium with long axis parallel to receptacle; penultimate cell transformed into probasidium. Fig. 5, two basidia borne on vertical basidiophore. Fig. 6, probasidium occurring as terminal member of vertical hypha. Figs. 7, 8, dikaryons in process of fusion. Fig. 9, spireme. Figs. 10, 11, later spiremes possessing polarity. Figs. 12, 13, spindles of first meiotic division. Figs. 14, 15, later stages of first division; deeply stained body on spindle between reorganizing nuclei. Fig. 16, second meiotic division; spindles parallel. Fig. 17, second division; spindles crossed; left end of lower spindle and right end of upper in higher plane; dark body at equator of lower spindle. Fig. 18, later stage of second division; areas occupied by spindles still staining deeply; spindles crossed. Fig. 19, four-nucleate basidium still lacking appendages. Fig. 20, basidium with appendages barely apparent; zygote nucleus undivided. Fig. 21, basidium with zygote nucleus once divided. Fig. 22, basidium with conspicuous appendages; zygote nucleus undivided. Figs. 23, 24, basidia partially drained of protoplasm; nuclei in passage to appendages. Fig. 25, appendages cut off from exhausted basal cell. Fig. 26, spireme in appendages; basidium in section showing convexity of septa at bases of appendages. Fig. 27, late stage of third division in (two) appendages; deeply staining body at equator of spindles; division in one appendage completed. Fig. 28, section of basidium with binucleate appendages; basal cell partially collapsed. Fig. 29, uninucleate appendage in process of putting out filament. Figs. 30-32, early stages in spore formation. Fig. 33, appendage nearly drained of protoplasm by spore. Fig. 34, nuclei in migration into spore; apparently first nucleus near middle of spore and second partly in filament, partly emergent. Fig. 35, immature spore, lateral upon filament, nuclei in opposite ends. Fig. 36, mature spore. Fig. 37, spore bearing nearly mature secondary spore. Fig. 38, spore bearing conidia. Figs. 39-43, *T. eichleriana*: Fig. 39, basidium with binucleate appendages cut off by septa. Fig. 40, appendages bearing young spores. Figs. 41-43, stages in production of secondary spore. Figs. 44-48, *T. lactea*: Fig. 44, young appendages, two with nuclei, upon partly exhausted basal cell. Fig. 45, basidium with longer, narrow appendages. Figs. 46, 47, binucleate appendages, with basal septum. Fig. 48, binucleate spore.

fewer or as many as eight—can be made out; these are to be interpreted as the chromosomes. There often appears to be a very minute black point at one or both poles; whether a centrosome is present there is not certain. The telophase of this division shows two deeply staining bodies, the reorganizing nuclei, lying close to the walls of the cell, with a dark line between them having a darker body midway between the two ends (figs. 14, 15). The newly formed daughter nuclei move somewhat away from the walls.

Apparently the formation of basidial appendages is not correlated with nuclear change; the first and even the second meiotic division may occur in a probasidium which shows no sign of developing appendages (figs. 10–19); or the zygote nucleus may still remain in a resting condition when the proliferations have reached a length one-third as great as that of the basal cell (fig. 22). The first indication of the rise of the appendages is little more than a slight flattening of the summit of the probasidium (fig. 20). Presently small knobs are formed (fig. 21). These swell distally without greatly increasing in diameter at the base, becoming short-stalked globes (fig. 22) and then, through elongation, pyriform bodies (fig. 23).

The nuclear spindles in the second meiotic division are transverse, either crossed at different levels near the summit of the basal cell (figs. 17, 18) or lying approximately parallel (fig. 16). The four nuclei formed lie near the apex of the basal cell until the appendages have attained considerable size (a length somewhat greater than half that of the basal cell), and by their increase in volume have partially exhausted the latter structure. The four nuclei thus come to be placed near the base of the appendages in a body of protoplasm that fills only the upper portion of the cell, most of the lower portion being occupied by a large vacuole (fig. 23). Finally the nuclei pass into the appendages, becoming somewhat elongated and more deeply stainable in so doing (figs. 23, 24); with them goes most of the remaining protoplasm. Apparently a film of protoplasm is left in the basal cell, since the latter does not collapse for some time (figs. 25–27).

As soon as the migration of the protoplasm into the appendages has been completed, septa are formed across the communicating passages, cutting off the appendages from the basal cell (figs. 25–28).

These septa can be made out clearly in the haematoxylin-phloxine preparations, the counterstain being strong enough to bring out the wall as a sharp pink line; they are even more marked in the preparations made with the Flemming triple stain. They are of course most evident in basidia which have been sectioned longitudinally (fig. 28). In the appendages thus cut off as separate cells a third mitosis then occurs. The spindles lie longitudinally; a late stage shows the same dark strand connecting the reorganizing nuclei, the same dark line in the equatorial position (fig. 27) that appeared in the meiotic mitoses. The daughter nuclei here formed approach each other somewhat; they may come to lie side by side in the cell or remain one above the other.

This division, like the earlier ones, is not correlated with change in the form of the cell (figs. 28, 29). But soon after mitosis has been completed the appendage has become narrower at the distal end and thus broadly spindle-shaped (fig. 29); a filament arises from the tapering apex (fig. 30). Elongation continues until the cell has reached a length half again as great as that of the probasidium; narrowing of the outer end continues, so that the appendage may become nearly conical (fig. 32). The basal cell by this time commonly has commenced to collapse; presently it has so far lost its earlier form as to become only a folded membrane lying at the base of the four walled-off appendages.

The spore first appears as a minute lateral swelling on the tip of the appendage (fig. 30). By the time it has attained half its final diameter the appendage has been so far exhausted of its protoplasm as to have much of the basal part filled with a large vacuole; the narrowing of the distal portion has progressed further toward the base (fig. 33). Presently the two nuclei pass into the spore, becoming much elongated and deeply stainable throughout as they pass through the very slender terminal portion of the filament (fig. 34). At first they lie one above the other (fig. 35); but later, and apparently always in the discharged spore, side by side near the blunt end (fig. 36). The mature spore is borne somewhat laterally upon the tip of the filament, the insertion of the latter being just beside the well developed apiculus of the spore. Living material studied in a moist chamber showed a drop of water held between the apiculus and

the filament, as figured for *T. fuscoviolacea* (fig. 52). This relation of filament and spore being precisely that given by BULLER (9, p. 498) as characterizing the sterigma and spore of Basidiomycetes whose spores are forcibly discharged, it must appear highly probable that such discharge occurs here; but none was observed.

The spore in germination produces most commonly a secondary spore, smaller, similar in form, borne at the tip of a short promycelium (fig. 37). Often, however, germination is by the production of numerous minute oblong conidia, about $1\ \mu$ long, borne on a slender, simple or branched filament (fig. 38).

TULASNELLA EICHLERIANA

In all characters except size, *Tulasnella eichleriana* is identical with the preceding species. The septum dividing appendage from basal cell is as clear as in the larger species, and mitosis within the appendage is as invariable (fig. 39). Only the one type of germination, by the production of a secondary spore, was observed (figs. 41-43).

TULASNELLA FUSCOVIOLACEA

In basidial cytology *Tulasnella fuscoviolacea* is identical with the preceding species, and in structure very similar. Here also the first postmeiotic division occurs in the appendages, after they have been separated by a wall from the basal cell (fig. 51). It differs from the two very similar species in the somewhat more globose form of the probasidium and appendages, in the allantoid spores, and in the greater size of all its parts. Material of two collections which seem to belong here shows fairly frequent clamp connections at the bases of the basidia (especially conspicuous on the young probasidia, fig. 49), and occasional clamp connections throughout the superficial mycelium (fig. 53). The occasional separation from the basal cell and subsequent functioning in spore production of the appendages, noted in this species by MARTIN (19) as in *T. deliquescens* by JUEL, could never be surely demonstrated from material touched by the sectioning knife; the regular collapse and disintegration of the basal cell before the formation of the spore indicates the probability of such an occurrence. In living material studied in a moist chamber the spores are borne in a lateral position upon their filaments; a

small, persistent water drop is held between apiculus and filament (fig. 52). Spore germination is by the production of a short promycelium, lateral or apical (figs. 55, 56), upon whose attenuated tip a secondary spore is borne. No conidia were observed.

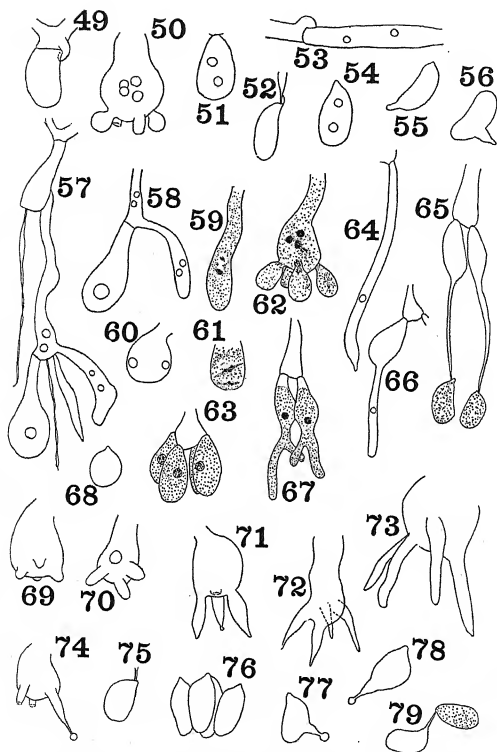
TULASNELLA LACTEA

The cytological development of *Tulasnella lactea* corresponds to that of the preceding species in all points, including mitosis in the appendages and formation of septa between basal cell and appendages (figs. 46, 47). The latter are grouped much more closely together than in the other species, upon the very summit of the basal cell (fig. 44). The texture and structure of the receptacle are notably different; it is composed of an intertwining mass, 30-50 μ in depth, of thick, irregular, vertical hyphae; on the tips of these threads are borne the small basidia.

TULASNELLA TULASNEI

In macroscopic and microscopic appearance *Tulasnella tulasnei* is quite distinct from the other species collected. It forms a continuous gelatinous-waxy layer, in places minutely tuberculate, in places smooth, much more strongly developed than the delicate bloomlike fructifications of the smaller forms. This layer deliquesces almost immediately upon maturity of the basidia, becoming a gelatinous slime, slightly undulate, finally perfectly hyaline.

The basidia are borne upon heavy vertical hyphae, commonly a number of them in a cluster upon one strong axis. Borne with the basidia upon such basidiophores, and upon the receptacle among them, are slender paraphyses (fig. 57) whose early deliquescence gives to the fruiting layer its gelatinous texture. The youngest basidia are clavate bodies (figs. 58, 59), often 20 μ long. These swell distally to form a broader portion (fig. 57) in which karyogamy and meiosis occur. The appendages arise as globose protrusions and enlarge to form oval bodies, which are then walled off at their bases (figs. 62, 63). In this species there is no third mitosis in the basidial appendage. Soon after formation of the septum there is thrust out from the appendage a filament which elongates greatly, often reaching a length greater than 20 μ before forming its spore (fig. 65). This



FIGS. 49-79.—FIGS. 49-56, *Tulasnella fuscoviolacea*: Fig. 49, probasidium with clamp connection at base. Fig. 50, four-nucleate basidium. Fig. 51, binucleate appendage with septum across base. Fig. 52, living spore upon filament, borne laterally, with water drop between apiculus and filament. Fig. 53, hypha of receptacle, with clamp connection. Fig. 54, binucleate spore. Figs. 55, 56, spores with promycelia. FIGS. 57-68, *T. tulasnei*: Fig. 57, vertical basidiophore bearing probasidium (at left), two half-

great length of filament appears to be an adaptation to the presence of the thick gelatinous matrix about the basidia. Often the basal portion of the appendage remains slender or becomes contracted, so that the diameter of the organ is about the same throughout its length (fig. 64). Frequent abnormalities found in this species, such as greatly attenuated, hypha-like appendages, often attaining a length of 100 μ and possessing protoplasm in only the distal third or quarter, or branching appendages (fig. 67), equally common, are also to be attributed to the gelatinous matrix. Spore germination is by secondary spore or by direct production of mycelium.

TULASNELLA ANCEPS

The mycelium of the fungus described as *Tulasnella anceps* is much like that of *T. violea* in general habit: loose, branching often at right angles, composed of short, thick cells. The basidia arise, like those of *T. violea*, as subglobose or pyriform cells, usually terminal upon the hyphae of the receptacle. From the summit of such a cell are thrust out commonly four rounded knobs (fig. 69) which elongate to form thick appendages of uniform diameter (fig. 70). The ends of these appendages then taper to a point, and often the diameter near the middle increases somewhat, before the formation of spores begins (figs. 71, 72). The mature basidium agrees in all details with the original description of BRESADOLA and SYDOW (6). Obviously no cytological observations were possible upon the material at hand; crush preparations showed clearly that no septa are formed at the bases of the basidial appendages (figs. 71-74). The spores regularly germinate by the formation of a similar secondary spore borne on a

disintegrated paraphyses, and young paraphysis not yet deliquescent; binucleate proliferation (at right), apparently a continuation of basidiophore. Fig. 58, young probasidia. Fig. 59, dikaryon in process of fusion. Fig. 60, probasidium after first division of zygote nucleus. Fig. 61, crossed spindles of second division; left end of lower spindle and right end of upper in higher plane. Fig. 62, basidium with nucleus in migration into appendage. Fig. 63, uninucleate appendages cut off by septum from exhausted basal cell. Fig. 64, elongated, slender appendage, uninucleate. Fig. 65, spores in place upon filaments of emptied appendages. Fig. 66, appendage with its single nucleus lying in filament. Fig. 67, appendages, uninucleate, with forked filaments. Fig. 68, spore. FIGS. 69-79, *T. anceps*: Figs. 69-74, basidia in various stages; appendages all lacking basal septa. Fig. 75, spore upon sterigma. Fig. 76, four spores clumped together. Figs. 77-79, spores in process of forming secondary spores.

short promycelium (figs. 77-79). Frequently the spores are clustered, lying parallel and in contact; such a group (fig. 76), especially when some of the spores have formed promycelia, greatly resembles a group of four tulasnellaceous basidial appendages; but no basal cell can be observed attached to them, and what appears to be the tapering distal end of an appendage proves to be the apiculus of a spore. Such grouping might result from the spores being borne close together on curving sterigmata, in the manner figured by BURT (11, p. 296) for *Corticium vagum*.

Discussion

JUEL's interpretation of the basidial apparatus of *Tulasnella* is perhaps the one most widely accepted. He gives the following as his grounds for regarding as basidiospores the oval bodies upon the basal cell:

(a) At a certain phase of their development—just before their germination—the form of these bodies is wholly sporelike.

(b) These bodies can fall from the basidium and germinate, although this occurs only exceptionally.

(c) The tubes that are attached to the upper parts of the sterigmata [!] vary considerably in length, even on the same basidium. . . . This applies less well to sterigmata, since they should be about equally long. But germ-tubes or promycelia have mostly no determinate length.

(d) These germ-tubes can at times arise at the base of the bodies interpreted by me as spores, and even exceptionally there can be two upon the same spore. Such an occurrence can hardly be explained by the earlier interpretation.

(e) The anomalous branching tubes figured are very strange if one interprets them as sterigmata, but according to my interpretation not at all notable.

(f) Cross-walls in the germ-tubes of germinating spores are known also in other fungi, and the structures figured can pass very well for germ-tubes, but less well for sterigmata.

(g) In *Muciporus corticola* [that is, *Tulasnella thelephorea*] each of the four nuclei found in the basidium divides as soon as it has passed from the basidium into one of the oval bodies. Since we interpret these bodies as spores, this nuclear division is not especially noteworthy. In a sterigma, however, that forms only a canal for the transfer of the basidial content to the spores, one would not expect nuclear division.

NEUHOFF's argument is based upon the resemblances of the *Tulasnella* basidium to that characterizing the Tremellaceae. Formation of a globose probasidium from which after a time are thrust

out commonly four thick tubes; production of spores after the basidial protoplast has been divided by walls into independent daughter protoplasts; germination of the spore by the formation on a promycelium of a similar secondary spore—these are tremellaceous characters, and tulasnellaceous as well. According to NEUHOFF's conception, that structure of the heterobasidium which the earlier workers called a sterigma is not homologous with the sterigma of the autobasidium; rather the basidium proper among the Tremellaceae possesses more or less the form of a hand and fingers. For the definitive sterigma, borne on the tip of the basidial extension, the epibasidium, NEUHOFF gives as a criterion that a migrating nucleus in passing through a sterigma assumes an elongated form and gives a staining reaction like that of chromosomes. This criterion is subject to the criticism that it may be only a response of the nucleus to the spatial relation, to be expected wherever a nucleus passes through a narrow opening (7, p. 9). But this very slenderness sets off the sterigma as a morphological unit from the epibasidium; and the criterion of function given by BULLER (8, p. 31), that "the typical sterigma . . . is to be regarded as an organ for the violent discharge of the spore," serves as a further indication of the validity of NEUHOFF's distinction.

The arguments which JUEL presents against the homologizing of the *Tulasnella* basidial appendages with sterigmata are not antagonistic to NEUHOFF's conception. The shape of the appendage (*a*, of JUEL's points) when it has reached its complete development and formed its spore is as much like that of the *Tremella* epibasidium as at an earlier stage it is sporelike. Its germination when separated from the basal cell (*b*) is essentially only what occurs in *Tremella*; in neither are the spores formed until the basidial protoplast is divided by walls into independent daughter protoplasts, each of which is then capable of producing a spore—the *Tulasnella* appendage even though separated from the exhausted basal cell, the *Tremella* basidial segment even though, as often occurs, the basidium may have become separated from the exhausted hypha on which it was borne. Somewhat similarly, in certain Ustilagineae (28) and in *Septobasidium retiforme* (13, p. 127), the epibasidium—a structure somewhat different, but like the appendage of *Tulasnella* in being only the

upper portion of the basidium—may form spores when separated from the hypobasidium. Variation in length (*c*) is the rule with *Tremella* epibasidia also (24, p. 195, pl. 10, figs. 9, 14, 26). Basal insertion of the filament, and the presence of two filaments (one basal), upon a single oval body (*d*), as figured by JUEL, are apparently geotropic responses to altered position of the basidium, and of no more morphological significance than what JUEL refers to as the lateral insertion of appendages, occurring wherever the long axis of a basidium lies horizontally. Branching of the apical filament (*e*) as figured by JUEL is no branching at all, but the lateral insertion of the sterigma; it occurs in *Tremella* (3, pl. 7, fig. 14 (3); 24, pl. 10, fig. 17, pl. 11, fig. 1). True branching does occur, however, in *Tulasnella* and in *Tremella* (18, pl. 6, fig. 14 *c*; 24, p. 196, pl. 12, fig. 9). Cross walls in the filament (*f*) may be accounted for by the development under water which JUEL gives as the cause of another abnormality of the same specimen, or perhaps even better by the gelatinous matrix in which the basidia lie imbedded; they have been noted in *Tremella* (24, pl. 10, fig. 16). Such hypha-like characters as septation, branching, and indefinite elongation have been observed by the writer to occur in the epibasidia of somewhat deliquescent specimens of *Tremella lutescens*. Mitosis within the appendage (*g*) is not the impossible behavior for an extension of the basidial cell that it would be for a sterigma.

Some of this has been said by NEUHOFF, and all of it is implied in his theory. His conception of the epibasidium provides for all the characteristics and abnormalities of the basidial appendages of *Tulasnella*, including their great similarities to the comparable organs of *Tremella*; it appears certain that this structure of *Tulasnella* is an actual epibasidium. The Tulasnellaceae are to be regarded as nearly related to the Tremellaceae, the two families being separated through the difference in position of the septa by which the basidial protoplast is finally divided. There appears no reason for questioning the assumption that the holobasidium (the *Tulasnella* basidium being such for this purpose) is nearer the ascus in morphology and phylogeny than the phragmobasidium. It must therefore be an acceptable hypothesis that the Tulasnellaceae, or forms very similar, are the ancestors of the Tremellaceae. The tremellaceous basidium may be taken to have arisen from the tulasnellaceous

basidium by the formation earlier in basidial ontogeny of the septa by which the basidial protoplast is divided.

The autobasidiomycete affinities of *Tulasnella*, perhaps equally strong, have been recognized in some degree by all investigators of this genus. HÖHNEL and LITSCHAUER gave them greatest weight, but failed to publish the list of transitional forms which justified to them the inclusion of *Tulasnella* among the Corticiaceae. However, such a series can be constructed, of *Corticium* species whose sterigmata show progressively greater length and thickness, in the final forms of the series resembling the greatly inflated epibasidia of *Tulasnella*. BOURDOT and GALZIN note as extreme forms, very close to *Tulasnella*, *Corticium flavescens* (Bon.) Massee, *C. sterigmaticum* Bourd., and *C. vagum* Berk. and Curt.

The fungus described as *Tulasnella anceps* Bres. and Syd. may be taken to be transitional. Its relatively enormous so-called sterigmata are usually broadest at the base, but frequently somewhat the widest midway between base and spore, being thus spindle-shaped. In their earlier stages they are blunt protuberances quite like the young epibasidia of typical *Tulasnella* species. But they never attain the width or the volume of the spore produced at their apex; they are never walled off from the basidial cell; they cannot be the organs "essential to the production of the spore"; they are not tulasnellaceous epibasidia, although very close to them. The formation of the basidia directly upon the loose web of hyphae that clings to the substratum is like that of the less gelatinous *Tulasnella* forms: the germination of the spore by the production of a secondary spore borne on a promycelium is a heterobasidiomycete character.²

² The taxonomic standing of *Tulasnella anceps* remains in question. BOURDET and GALZIN list it as a synonym of *Corticium vagum* Berk. and Curt. Its basidia agree well with those of the latter species, and the frequent clumping of its spores would be most easily brought about by their being borne in the manner shown by BURT for *C. vagum*. But in the somewhat meager material of *C. vagum* available for comparison, no spores show the germination by renovation characteristic of SYDOW's specimens. However, the *C. vagum* studied, growing as it did on potato stems, would have been placed by BOURDOT and GALZIN in *C. solani* (Prill. and Delacr.) Bourd. and Galz. The problem is complicated by this separation, which BURT (11, p. 295) does not recognize. In any event, the fungus distributed by SYDOW is not a *Tulasnella*, and cannot be taken, as suggested by CLEMENTS and SHEAR (12), to be the type of the genus. BOURDOT and GALZIN identify SCHROETER's species with *T. violea* (Quél.) Bourd. and Galz.; since the identity of the TULASNES' fungus cannot certainly be determined, *T. violea*, published in 1882, must be the type.

The nature of this fungus, and of other similar species among the simplest corticiums (one other whose spores germinate by renovation), suggests the further hypothesis that *Tulasnella* is the ancestral form of *Corticium*, and through it, of the autobasidiomycetes. The autobasidium may be taken to have arisen by the loss of the septum across the base of the epibasidium and subsequent telescoping of the epibasidium, the sterigma finally coming to be seated upon the basal cell.

Although JUEL's arguments do not demonstrate the homology of the *Tulasnella* basidial appendages with basidiospores, yet they are valid enough as indications of the sporoid nature of the bodies in question. The existence of a septum across the base of the epibasidia, a structure which JUEL failed to observe (16, plate), and the supposed absence of which is the one basis of RAUNKIAER's criticism of JUEL's theory, is an even more cogent argument here. In discussing this family, GÄUMANN and DODGE (14) state:

If the basidium is conceived as developing from an ascus by gradual exogenous spore production, perhaps *Tulasnella* represents a transitional stage where the spore mass, without secreting a wall about itself, is pushing out of the gonotocont without having reached the stage of a separate entity before it germinates. . . . On the other hand, such a conception would indicate that it had not yet reached a suitable mechanism for spore discharge, having lost that of the ascus without attaining that of the basidiospore.

This suggestion embodies just such a conception as will account for the sporoid characters of the *Tulasnella* epibasidium. It falls short of adequacy in accepting that the body in question has not "reached the stage of a separate entity," in denying to the fungus "a suitable mechanism for spore discharge," and in homologizing the oval bodies upon the *Tulasnella* basidium with basidiospores.

The cytological homologies between basidium and ascus, and between basidiomycete dikaryon hypha and ascogenous hypha, make derivation from the Ascomycetes the one acceptable theory of the rise of the Basidiomycetes. Such derivation may well be taken to be from such a form as *Ascocorticium* (5, pp. 145, 146), whose general structure resembles that of the simpler resupinate basidiomycetes. If in some such fungus ascospores, in place of being discharged in the jet, were to be merely thrust out in pockets of the acsus wall, a

transitional form leading toward the basidium would there be achieved. If then the ascospore protoplast, its wall failing of formation, were to be surrounded rather by the ascus wall of the pocket and a septum formed across its base, the body thus produced, containing the haploid nucleus, although not actually a spore, would be capable of exhibiting such sporoid characters as JUEL lists; it would be just such a body as the *Tulasnella* epibasidium. Ascospores are known to germinate by the formation of conidia on a short promycelium (25, p. 177; 26, pl. 16, fig. 19); such a conidium produced in the germination of the undischarged ascospore protoplast might well be taken to be the ancestor of the basidiospore. A similar interpretation was advanced by BESSEY (1), who, homologizing the segments of the rust basidium with ascospores, termed the basidiospores secondary spores. Whether for a time in the phylogenetic history of these forms the walled-off appendages may have fallen from the basal cell, germinating subsequently, or whether germination upon the basal cell came at once, at present cannot even be conjectured and seems not essential to the hypothesis. Similarly, there are not sufficient grounds for indicating the relations among the 5-8-spored species of *Tulasnella*, the 4-spored species with mitosis in the epibasidium, and the 4-spored species without such mitosis. The post-meiotic division occurring before basidiospore production may represent the delayed third division of the zygote nucleus—a conception admissible here, since the present *Tulasnella* epibasidium is certainly not an ascospore—or a step in ascospore germination.

Summary

1. Following karyogamy and the two meiotic divisions, the probasidium of *Tulasnella* produces from its summit commonly four inflated epibasidia; into these migrate the nuclei and protoplasm of the basal cell; when the latter has been exhausted the epibasidia are separated from it by septa across their bases. In *T. violacea*, *T. fuscoviolacea*, *T. eichleriana*, and *T. lactea* a third mitosis occurs in the epibasidia; following this each epibasidium elongates and upon a filament produced from its apex bears a binucleate basidiospore. In *T. tulasnei* there is no mitosis in the epibasidia.

2. Similarities existing between the *Tulasnella* basidium and that of the Tremellaceae argue the close relation of the two types; the tremellaceous basidium may be regarded as having evolved from the tulasnellaceous by the formation of basial septa at an earlier stage in basial ontogeny. Similarly the existence of transitional forms (among them the fungus described as *T. anceps*) argues the close relation of the *Tulasnella* basidium to that of *Corticium*; the auto-basidium may be regarded as having evolved from the tulasnellaceous type by loss of the epibasidium. The sporoid characters of the *Tulasnella* epibasidium strongly suggest its evolution from the ascospore; consequently the epibasidium may be taken as the true homologue among the basidiomycetes of the ascospore, and the basidiospore as the homologue rather of an ascospore germination-conidium.

This study was undertaken at the suggestion of Professor G. W. MARTIN and completed in the mycological laboratory of the University of Iowa under his supervision.

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ECOLOGICAL RELATIONSHIPS OF *PINUS* *SABINIANA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 438

GEORGE W. GRAVES

(WITH FOURTEEN FIGURES)

Introduction

As one ascends the Sierra Nevada from the west, the most conspicuous feature of the landscape of the foothills and the lower mountain slopes at about the 600-foot level is *Pinus sabiniana*, known locally as the Digger pine. It is scraggly and irregular in growth, attaining a height of from 40 to 90 feet, and frequently dichotomously branched. This species occurs in open stands, reaching in more favorable situations the status of a thin open forest. Its zonal distribution is interesting, for it occurs only between 600 and 4000 feet elevation, with a peculiar gap in its range south of the Kings River. It is widely distributed in California on the interior coast ranges and the western slope of the Sierra Nevada.

The ecology of the area under discussion has been studied by various investigators. JEPSON (5) discusses the geographical distribution of the seed plants in California. COOPER (3), in his extensive treatment of the broad sclerophyll vegetation of the same state, considers the Sierra Nevada woodlands and chaparral although studying more in detail the coastal and southern sclerophylls. KLYVER (6) has recently mapped the plant associations in a transect 7 miles wide by 80 miles long across the Sierra Nevada. This transect covers the northern edge of the area discussed in this paper. The Forest Service is studying the woodlands and chaparral in connection with other vegetation types in relation to fire. Papers by SHOW and KOTOK (11, 12) treat this phase of the subject.

Species of the woodland and chaparral in this and other parts of the state are considered in papers by CANNON (1, 2), PLUMMER (8), MILLER (7), and STERLING (10).

Investigation

VEGETATION TYPES

The vegetation types are arranged in the order in which they are encountered in ascending the mountains from the San Joaquin Valley floor. A more detailed list of species is given under the discussion of succession.

GRASSLAND.—The San Joaquin Valley floor is largely under cultivation, either by dry farming or by irrigation, except in those areas where an accumulation of alkali makes cultivation impossible or too expensive. The climatic conditions prevailing and what relict evidence can be obtained indicate that the predominant association was of a bunch grass type dominated by *Stipa pulchra* which is now restricted to small isolated areas. Where land is not cultivated or where cultivation has been abandoned, the original grasses have been largely replaced by a variety of introduced grasses and herbs.

Tree species occur only along the water courses or the floodplains of the Kings and San Joaquin rivers. These rivers have built up deltas of considerable size, and in overflow areas and along modern irrigation ditches hydric associations of *Typha*, *Scirpus*, *Anemopsis*, etc., are found. Habitats high in salt content are dominated by *Distichlis*, *Sporobolus*, *Salicornia*, *Allenrolfea*, *Sarcobatus*, etc.

OAK SAVANNA.—In contact with the grassland of the valley floor and the frontal foothills is the oak savanna. It begins just inside the frontal foothills at an elevation of about 500 feet. In its lower portion it is open and parklike, with the trees at wide intervals. These are limited to *Quercus douglasii*¹ and *Q. wislizenii*, and layer shrubs as *Ceanothus cuneatus* and *Arctostaphylos mariposa* are restricted in distribution. In isolated rocky outcrops close to the crest of the frontal foothills small associations of *Rhamnus californica* are found.

Throughout the woodland zone there is a great uniformity of species. The grasses and herbaceous plants bloom early, forming in many cases aspect societies of great beauty. The period of bloom is very short and during the rest of the year the hillsides are dry and brown, except for the grey-green of the sclerophyllous plants.

¹ Plant names are from JEPSON (5).

The following species are distributed throughout the area of the oak savanna:

Shrubs and trees of oak savanna

STREAM BANKS

Cephalanthus occidentalis
Platanus racemosa
Populus fremontii
Salix nigra
S. sessilifolia var. hindsiana

OTHER SITUATIONS

Arctostaphylos mariposa
Ceanothus cuneatus
Lotus scoparius
Nicotiana glauca
Quercus douglasii
Q. wislizenii
Rhamnus californica

Grasses and herbs forming conspicuous societies

Amsinckia douglasiana
Avena barbata
A. fatua
Baeria chrysostoma
Brodiaea capitata
B. ixioides
B. laxa
Calandrinia caulescens
Gilia capitata
Godetia amoena
Heliotropium curassavicum

Layia platyglossa
Lupinus albifrons
L. densiflorus var. lacteus
L. formosus
Madia elegans
Montia perfoliata
Phacelia californica
Plagiobothrys canescens
P. nothofulvus
Platystemon californicus
Trifolium tridentatum

Grasses and herbs of general distribution

Asclepias californica
A. cordifolia
Athyranus unilateralis
Brodiaea coronaria
B. hyacinthina
B. volubilis
Bromus hordeaceus
B. rubens
B. tectorum
Calochortus luteus
C. venustus
Calyptidium umbellatum

Gilia tricolor
Hordeum murinum
Juncus balticus
J. mexicanus
Lathyrus nevadensis
L. sulphureus
Layia glandulosa
Lepidium nitidum
Limnanthes douglasii
Linanthus bolanderi
L. dichotomus
L. serrulatus

Chaenactis glabriuscula	Mimulus guttatus
Cirsium occidentale var. coulteri	Monardella candicans
Collinsia bicolor	Plantago erecta
Cotyledon laxa	Raphanus sativus
Cryptantha flaccida	Rumex acetosella
Delphinium decorum	R. crispus
Diplacus longiflorus	Salvia columbariae
Eschscholtzia californica	Sanicula bipinnatifida
E. lobbiai	Senecio douglasii
Festuca megalura	Silene gallica
Galium aparine	Silybum marianum
Gilia gilioides	Sisyrinchium bellum
Lotus subpinnatus	Solanum xanthii
Mentzelia lindleyi	Stellaria media
Mimulus bicolor	

DIGGER PINE-OAK WOODLAND.—At an elevation of about 600 feet the oak savanna woodland merges into the Digger pine-oak woodland. It differs from the vegetation just below it in the conspicuousness of *Pinus sabiniana*. Although the grasses and herbs are an important phase, the trees in many cases form an open sclerophyll forest. Associations of layer shrubs such as *Ceanothus*, *Arctostaphylos mariposa*, *Rhus diversiloba*, and *R. trilobata* are common, as are treelike shrubs such as the deciduous *Aesculus californica* and *Cercis occidentalis*. In many cases this woodland becomes a brushland as it grades into hard chaparral just above it.

In addition to the species listed for the oak savanna, the following are characteristic of the Digger pine-oak woodland.

Shrubs and trees of the Digger pine-oak woodland

STREAM BANKS	OTHER SITUATIONS
<i>Alnus rhombifolia</i>	<i>Aesculus californica</i>
<i>Fraxinus dipetala</i>	<i>Berberis californica</i>
<i>F. oregona</i>	<i>Ceanothus divaricatus</i>
<i>Salix lasiandra</i>	<i>Cercis occidentalis</i>
	<i>Cercocarpus betuloides</i>
	<i>Pinus sabiniana</i>

Shrubs and trees of the Digger pine-oak woodland—Continued

VINES	OTHER SITUATIONS
Echinocystis macrocarpa	Rhamnus crocea
Lonicera interrupta	Rhus diversiloba
Vitis californica	R. trilobata
	Sambucus velutina

Grasses and herbs of general distribution

Allium spp.	Nemophila menziesii
Clarkia elegans	Orthocarpus erianthus
Dodecatheon hendersonii	Panicum hirticaule
Eleocharis palustris	Potentilla glandulosa
Galium trifidum	Saxifraga virginensis var. californica
Hemizonia wrightii	Scrophularia californica
Lotus scoparius	Thysanocarpus curvipes
Lupinus stiversi	Trifolium ciliatum
Nemophila maculata	

CHAPARRAL.—The term chaparral is used in various ways. JEPSON (5) calls the type of chaparral occurring here the "hard" type to distinguish it from the "soft" type which is a shrub formation found in the Transition Zone. The practically pure stands of *Adenostoma fasciculatum* which cover large areas in southern California, on the Coast Range and on the foothills of the Sierra Nevada farther north are also called chaparral. *Adenostoma*, however, has not been collected in this area.

Hard chaparral is comparable with the maquis of the Mediterranean region. It is a mixed formation, with many tree species adapted to the xerophytic conditions, and has undoubtedly been greatly influenced in extent and structure by fire. Since the term chaparral has become established it will be used in this study to denote this particular type.

The chaparral then, ranging in elevation from 2000 to 4000 feet in this area, is a dense, almost impenetrable thicket, woody and evergreen except for a few deciduous forms, the most notable being the distinctive *Aesculus californica*. *Aesculus* adapts itself to its xerophytic situation by an early production of leaves and flowers. By the middle of June its leaves are dry and brown and the trees appear as brown blotches on the hillsides.

The most common genera of the chaparral are *Quercus*, *Ceanothus*, *Arctostaphylos*, *Umbellularia*, *Fremontia*, and *Aesculus*, with frequent specimens of *Pinus sabiniana*.

Because of the density of the stand, grasses and herbs occur sparingly in the mature chaparral. A list of species that have invaded the chaparral following fire is given under the discussion of succession in relation to fire.

Shrubs and trees of the chaparral

<i>Aesculus californica</i>	<i>Prunus subcordata</i>
<i>Arctostaphylos mariposa</i>	<i>Ptelea baldwinii</i> var. <i>crenulata</i>
<i>Berberis californica</i>	<i>Quercus chrysolepis</i>
<i>Calycanthus occidentalis</i>	<i>Q. douglasii</i>
<i>Carpenteria californica</i>	<i>Q. wislizenii</i>
<i>Ceanothus cuneatus</i>	<i>Umbellularia californica</i>
<i>C. divaricatus</i>	<i>Rhamnus crocea</i>
<i>C. integerrimus</i>	<i>R. californica</i>
<i>Cercis occidentalis</i>	<i>Rhus diversiloba</i>
<i>Cercocarpus betuloides</i>	<i>R. trilobata</i>
<i>Eriodictyon californica</i>	<i>Sambucus velutina</i>
<i>Fremontia californica</i>	<i>Staphylea bolanderi</i>
<i>Pinus sabiniana</i>	<i>Styrax officinalis</i>

FOREST.—The forest is of the yellow pine type which grades in more mesophytic situations and at higher altitudes into the mixed conifer type. *Pinus ponderosa* is the common and most conspicuous conifer, and associated with it are *Libocedrus decurrens* and *Quercus kelloggii*. The forest area in contact with the chaparral has been both logged and burned, but the stand of yellow pine is extensive and is reproducing. Layer shrubs of *Arctostaphylos mariposa* and *Ceanothus integerrimus* are common. The ground cover is *Chamaebatia foliolosa*.

The area studied in detail is between the San Joaquin River on the north and Kings River on the south, some 25 miles wide and extending from the San Joaquin Valley floor, 300 feet in elevation, to about 4000 feet, the highest elevation at which *P. sabiniana* is found in this area.

Pinus sabiniana, one of the nut pines, is described by JEPSON (5) as 40 to 90 feet high, the trunk in typical trees parting

into a cluster of erect branches which form a broomlike top. It is found on arid foothills, forming a very thin stand on Sierra foothills and coast ranges, mostly toward the interior, reaching the coast only in the Santa Lucia Mts. The timber is inferior.

JEPSON (4) states that *P. sabiniana* inhabits the arid foothills of the Sierra Nevada and coast ranges surrounding the oval of the Great Valley of the Sacramento and San Joaquin, save for a curious break of 20-25 miles on either side of the Kaweah River in the southern Sierra Nevada between the Kings River and White River. It is also generally distributed throughout the dry valleys and hills of the north coast ranges as far westward as the borders of the Redwood belt. In the south coast ranges it is common in the Mt. Diablo, Mt. Hamilton, and San Carlos ranges, eastern slope of the Santa Cruz and Santa Lucia Mts., then extends southward to the Mt. Pinon region in Ventura County, and finally to the Sierra de la Liebre south of Tehachapi and Antelope Valley, the southernmost locality.

In ascending the foothills, aside from the stream bank associations dominated by *Populus fremontii*, *Salix nigra* var. *vallicola*, and *Platanus racemosa*, the first trees seen are *Quercus wislizenii*, found on rocky outcrops and along the fissures of the lava caps of the local table mountains, and *Q. douglasii*, frequently in pure stands in level places but tending to occupy the lines of seepage between the rolling frontal foothills. Above the 600-foot level *Pinus sabiniana* appears, and with them forms the dominant tree vegetation of the Upper Sonoran, or area of woodland and chaparral under discussion.

Within its zone of distribution, *Pinus sabiniana* occurs in practically every situation. Not only on the dry hillsides, but approaching close to the banks of perennial streams, with excellent stands on the floodplains of the Kings River and on the banks of the San Joaquin above Friant, the Digger pine is universal.

TOPOGRAPHY AND SOILS

The area selected for intensive study is typical of the southern Sierra Nevada and is situated between the San Joaquin and Kings rivers. Its elevation is from about 600 feet on the low frontal foothills to 4000 feet, the upper limit of the Digger pine in this area.

The general direction of the Sierra Nevada is northwest to southwest, with the foothills having a gradual slope westward and southwestward. The two main rivers debouch from the foothills at an elevation of about 400 feet. They have built up deltas of considerable size at the edge of the foothills and on the floor of the San Joaquin Valley, but a study of their vegetation is not part of this paper. The frontal foothills, which are of the Tertiary and Quaternary periods, have been eroded to a generally rounded appearance with no vegetation except grass and herbaceous material.

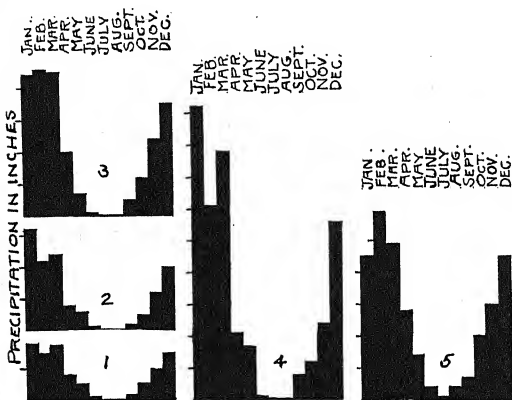
The core of the Sierra Nevada is granite, with a remnant of metamorphosed sedimentaries on the western flank, some remnants on the high crests of the range, and great blocks of sedimentaries abutting against the fault scarps to the east. Aside from relatively small amounts of basalt along the San Joaquin River, therefore, the bulk of the soil-building material is from the weathering of granite rocks. The soils of the brushland and chaparral areas are largely granitic. With increased altitude and precipitation there is an increase in humus, particularly at the upper levels of the distribution of the Digger pine. Local topography with drainage into low spots, coupled with the action of intermittent streams, has in some places caused an accumulation of soils rich in humus; but in general the soils are light and sandy with good drainage.

CLIMATE

The Great Valley of California, whose northern and southern divisions are known as the Sacramento and the San Joaquin valleys respectively, has an average width of about 50 miles. The climate is characterized by its rainless, hot summer and mild winters with light rainfall. There is a slight increase in precipitation from the west to the east side of the valley. With slight modifications, this general characterization applies to the foothill area. The moisture-laden winds from the west are cooled by the Coast Range with resulting precipitation. The eastern slopes of the coast ranges are more xerophytic than the western slopes. The greater height of the Sierra Nevada in relation to the coast ranges enables it to receive enough moisture at higher elevations to support a xero-mesophytic

coniferous forest, while its lower western foothills and eastern slopes are decidedly xerophytic.

In figures 1-5 the distribution of precipitation is shown over a period of years. The woodland and chaparral area extends from an



FIGS. 1-5.—Average monthly distribution of precipitation in inches from January to December at five stations on western slope of Sierra Nevada Mts.: Fig. 1, Fresno, elevation 290 feet, precipitation 9.82 in., 41-year record; grassland. Fig. 2, Friant, elevation 345 feet, precipitation 12.81 in., 24-year record; grassland-oak savanna. Fig. 3, Auberry, elevation 2050 feet, precipitation 23.49 in., 13-year record; Digger pine-oak woodland, chaparral. Fig. 4, Northfork, elevation 3000 feet, precipitation 36.85 in., 15-year record; Digger pine-oak woodland, chaparral. Fig. 5, Big Creek, elevation 4900 feet, precipitation 30.88 in., 13-year record; coniferous forest.

elevation of about 500 feet, just above Friant, to an elevation of approximately 4000 feet at a point above Auberry, depending upon how far fire has driven the yellow pine back and permitted dominance of the chaparral. The precipitation records from Friant, Auberry, and Northfork indicate typical distribution by months in the brushland and chaparral.

Comparing these stations (for all of which records are available for periods of from 13 to 41 years) with Fresno on the floor of the San Joaquin Valley below and Big Creek in the mixed conifer zone above, the distribution of precipitation over the year is comparably uniform, with the maximum in January-March. There is a rapid decrease in April, May, and June; no precipitation or only a small amount at the higher stations during the summer months; and a

TABLE I

AVERAGE MEAN MINIMUM (A) AND AVERAGE MEAN MAXIMUM (B) TEMPERATURES
BY MONTHS FOR FIVE STATIONS FROM FLOOR OF GREAT VALLEY
TO MIXED CONIFER ZONE

STATION	ELEVATION (FEET)	° F.												No. OF YEARS
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Fresno	300	A.. 38.0	41.2	44.2	47.3	52.0	59.0	64.6	63.4	57.9	50.6	43.0	38.2	35
		B.. 54.3	60.9	65.5	73.3	80.9	91.2	99.1	97.6	89.1	78.2	66.1	54.7	35
Clovis	400	A.. 33.3	36.6	39.3	43.6	48.9	56.1	61.5	59.3	53.9	46.1	38.3	34.9	13
(near)		B.. 55.2	62.3	67.4	74.2	85.5	95.6	102.8	100.1	93.8	84.2	67.1	55.8	13
Auberry	2050	A.. 31.6	35.3	38.8	42.3	48.4	57.3	62.9	61.2	53.8	44.7	37.2	32.9	14
		B.. 54.9	57.8	60.7	67.0	76.9	86.9	95.1	93.5	86.6	74.9	65.0	57.9	14
North- fork	3000	A.. 29.7	31.8	34.8	38.3	42.4	50.4	57.5	55.4	49.2	41.5	34.8	29.8	21
		B.. 54.7	56.7	60.7	66.7	77.5	82.5	94.5	92.4	85.3	75.0	65.5	55.7	21
Big Creek	4900	A.. 31.8	32.8	34.3	38.4	45.9	54.3	61.7	59.9	54.2	46.4	40.2	34.6	14
		B.. 49.4	50.5	53.5	57.9	66.0	75.6	83.9	82.5	76.2	66.4	58.7	51.2	14

gradual increase in September, October, and November to the maximum again.

Temperature records (table I) are available for a rather long period of years for stations covering this area from the valley floor to well above the zone of woodland and chaparral. The difference between the average mean minima and the average mean maxima during the period of lowest precipitation is rather uniform for the different stations, but the average mean maxima are, as expected, highest at Fresno and Clovis, on the valley floor and the frontal foothills. They are rather uniform for the Auberry and Northfork stations at elevations of 2050 and 3000 feet, and decrease sharply above these points. This seasonal distribution of precipitation, with the maxi-

imum during the winter and a dry summer accompanied by high temperatures, provides the environmental complex for the development of broad sclerophylls as discussed by SCHIMPER (9) and COOPER (3).

Figures 1-5 indicate that, although maximum precipitation occurs at both Fresno and Big Creek over a period from September to March, the bulk of it is in the four months from December to March with practically none during June, July, and August. Precipitation in the San Joaquin Valley is therefore sufficient to support only grassland, while that of the higher elevations permits the development of coniferous forests, the precipitation of the winter months being sufficient to meet the losses of the dry growing season. The sclerophylls of the intermediate points in the woodland and chaparral are so adapted as to take advantage of any favorable period for growth when moisture and temperature conditions are suitable.

INSTRUMENTAL DATA

In order to obtain data on certain environmental factors, particularly the rate of evaporation in relation to the distribution of *Pinus sabiniana*, atmometers were established at four different points and maintained from April 20 to November 16 during the season of 1929, and from April 5 to October 14 during the season of 1930. Stations were located so as to have one representative of the valley grassland, one representative of the grassland-oak savanna, one representative of the Digger pine-oak woodland, and the highest station at the advancing edge of the chaparral into the yellow pine coniferous forest area.

STATION NO. 1, FRESNO, ELEVATION 300 FEET.—Conditions here are representative of the floor of the San Joaquin Valley. The location was in the college garden and somewhat east of the center of the valley on an east to west line. Although not an ideal location, as neighboring buildings interfered somewhat with the free movement of air currents, yet it seemed the only practicable location from standpoint of accessibility and protection for the atmometers.

STATION NO. 2, FLEMMING RANCH, ELEVATION 450 FEET.—This station was located in the hills at a point in well established oak savanna. *Quercus douglasii* and *Q. wislizenii* are common, but *Pinus*

sabiniana does not occur so far down. The station was some 75 feet from an intermittent stream which usually runs for a few weeks in the spring. Along the stream is a scattered stand of *Populus fremontii* and *Platanus racemosa*. The topography is broken, but a level spot was located, and fenced to prevent depredations by stock. Shrubs and trees immediately around the station are not reproducing, owing to grazing, but on the hills above they are plentiful.

The soil at this station is higher in humus than at any of the other stations except no. 4. An area of metamorphosed sedimentary rock on the flanks of the Sierra Nevada is exposed at this point, which combined with general topography and previous stream action has produced a soil somewhat superior to those of the area as a whole.

STATION NO. 3, HUGHES RANCH, ELEVATION 700 FEET.—*Pinus sabiniana*, as well as *Quercus*, *Ceanothus cuneatus*, and *Aesculus californica*, are well established. Seedlings of *P. sabiniana* and *Ceanothus* are developing within a short distance of the atmometers. In order to find an open spot away from cultivated land and free from shade, it was necessary to locate the atmometers on a slight ridge. The soil of this ridge is a shallow layer of decomposed granite; in fact it was most difficult to get post holes sufficiently deep to put up an adequate fence. The area is representative of the Digger pine-oak woodland.

STATION NO. 4, YELLOW PINE FOREST, ELEVATION 4000 FEET.—This site was selected because of its convenience to the road and its location at the line of contact between the chaparral and the yellow pine forest. Scattered stands of *Pinus sabiniana* occur 25 yards below this station and a few occur above. The general exposure of the ridge upon which the station was located is to the west, and the high altitude of the yellow pine conifer forest cover at this point is undoubtedly due to the encroachment of chaparral following fire. Although *P. ponderosa* is the most common conifer, the typical trees and shrubs of the Transition Zone are present. *Libocedrus decurrens*, *Abies concolor*, and *Quercus kelloggii* occur, while the characteristic *Chamaebatia foliolosa* is the ground cover.

ATMOMETER PROCEDURE

At each station the atmometers were located at ground level with the bulbs about 14 inches above the surface of the soil. They were

situated so as to avoid shade so far as possible, but at station no. 4 shade could not be entirely avoided.

The atmometers were of the standardized sphere type fitted with a Livingston-Thone rainproof valve. During the season of 1929 they were run in pairs, but during the 1930 season black and white spheres were maintained at each station.

Because of the distance from Fresno, it was possible to read the atmometers only fortnightly during the 1929 season, but during the 1930 season they were read weekly throughout most of the period. At each visit the bottles were exchanged for fresh ones containing a known weight of water and the old bottles reweighed to determine the amount of water lost. This proved a more practicable method for field work, in view of the high water loss, than to add water by means of graduates and pipettes. At each visit during 1930 the temperature at the surface of the ground was taken by laying a thermometer on the ground and covering the bulb lightly with trash. An attempt was made to collect soil samples at stations no. 2, 3, and 4 at a depth of 18 inches, but owing to the great variation of the soils of the different stations and the extremely rocky nature of the country, uniformity in sampling was difficult. The soils were collected in tight cans and dried in the laboratory at 105° C. The temperature of the soil at 12 inches' depth was also taken by means of a thermometer, either by lowering it in an auger hole where the ground was hard or by plunging it in soil at that depth when it could be removed by shovel.

ENVIRONMENTAL FACTORS

Even to the most casual observer, the marked zonation and grouping of the trees and shrubs is evident as one ascends the foothills from the San Joaquin Valley floor. It is difficult to obtain definite data bearing on this distribution, and particularly to interpret such data, since the problem is complicated by many factors.

The station at Northfork is some 15 miles to the north of the area studied, but conditions of topography and vegetation are identical with the corresponding elevation under discussion. A summary of the available temperature data is given in table I. The outstanding features are the high summer temperature and the wide range between maxima and minima.

The uniformity in the distribution of precipitation has already been noted (figs. 1-5), there being an increase with altitude but the greatest precipitation coming in the same months at higher stations as at the lower stations. It should be pointed out that there is not a direct and uniform increase with altitude but an increase which reaches a maximum and then diminishes with greater increase in altitude.

Considering temperature alone, increase in altitude produces a uniform decrease in the average mean temperature, but as between

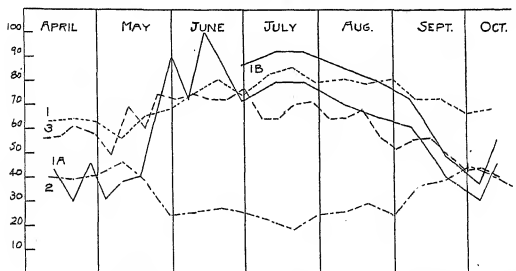


FIG. 6.—(1) Comparison of mean daily temperatures ($^{\circ}$ F.) at Fresno: evaporation per day in grams from (1A) white spheres and (1B) black spheres; (2) relative humidity; and (3) solar and sky radiation in gram-calories per sq. cm. of horizontal surface; season 1930.

the San Joaquin Valley and the Digger pine-oak woodland, it is a matter of but a few degrees and not sufficient to be a determining factor in distribution. In fact *Pinus sabiniana* has been planted in many places, not only on the valley floor but in the area to the south of Kings River, where it is conspicuously absent in the native vegetation. In all cases it seems to be thriving, with every indication that the life span will be the same as in the area of natural distribution.

In figure 6 the relations of mean daily temperature, relative humidity, solar and sky radiation, and evaporation from white and black atmometer spheres are shown. Although data on humidity and radiation are available only for the valley floor, other data of tempera-

ture and rainfall indicate that comparative conditions exist in the Digger pine-oak woodland of the foothills.

It is to be noted that July, the month of highest temperatures, shows the lowest humidity; while in June occurred a period of high evaporation from the white spheres. June was also the month of highest solar and sky radiation. Black spheres were available from July to the end of the season, and show a parallel increase in water loss as compared with the white spheres.

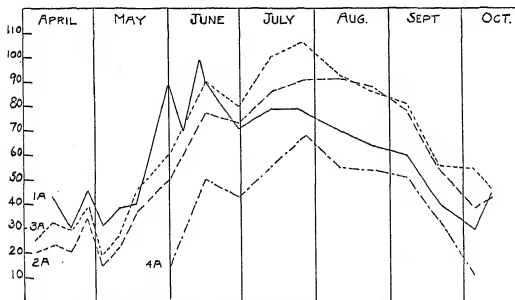


FIG. 7.—Comparison of rate of evaporation per day in grams from white spheres at stations 1, 2, 3, and 4; season 1930.

The relative rate of evaporation from white spheres at all stations as shown in figure 7 indicates that conditions in relation to water loss are more severe on the valley floor in the early summer, but in July conditions in the Digger pine-oak woodland exceed those of the valley floor as well as the oak savanna of the first foothills. This great increase in the evaporating power of the air during July is difficult to explain. In the matter of temperature and precipitation, as all available records show, conditions in this zone are more favorable than in the zones below.

Significant features of the dominant elements of the foothill vegetation, the oaks and the Digger pine, are their tolerance to light and high temperatures and their capacity to develop in spite of the extremely desiccating conditions during the summer months.

Some evidence as to the amount of radiant heat absorption by an evaporating surface is shown in figure 8 by the relative loss from white and black atmometer spheres. Considerable difficulty was experienced in maintaining the black atmometers. They seemed to

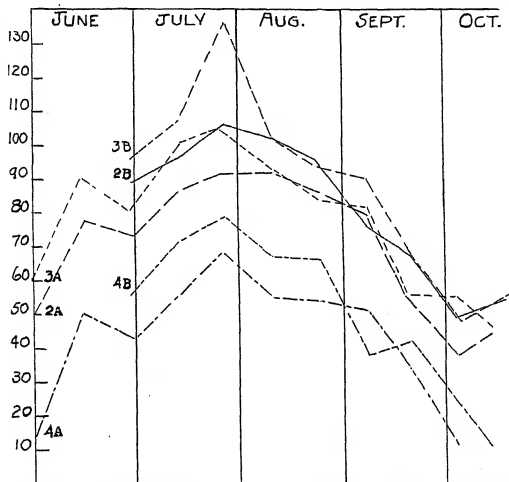


FIG. 8.—Comparison of rate of evaporation per day in grams from (A) white spheres and (B) black spheres at stations 2, 3, and 4; season 1930.

be especially attractive to rodents, who scratched off more or less of the lamp black. All stations showed an increased water loss in the black spheres, especially through June and July, with a marked decrease in the difference between white and black spheres in August and September. The greatest increase in evaporation is at station no. 3 in the Digger pine-oak woodland, and occurred in July.

The influence of ground temperature, soil temperature, and percentage of soil moisture upon the distribution of *Pinus sabiniana* is difficult to interpret. Within its altitudinal range and in the area

under discussion, *P. sabiniana* is found in every sort of situation, from the banks of perennial streams to the very unfavorable conditions of granitic outcrops, basaltic talus slopes, and the slightly weathered tops of basaltic table mountains.

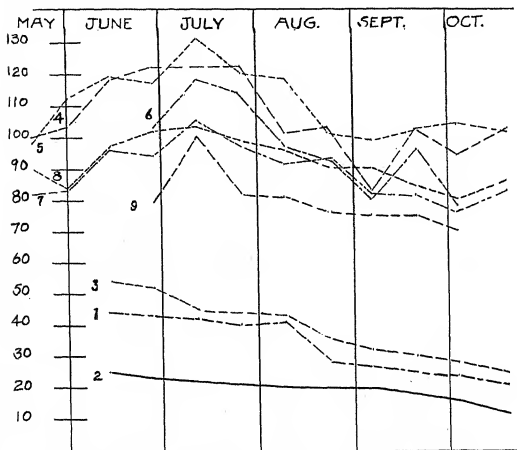


FIG. 9.—Comparison of percentage of soil moisture and surface and soil temperatures (° F.) at stations 2, 3, and 4; season 1930: 1, 2, 3, percentage of soil moisture at stations 2, 3, and 4; 4, 5, 6, surface temperatures at stations 2, 3, and 4; 7, 8, 9, soil temperatures at stations 2, 3, and 4.

The data assembled in figure 9 upon ground and soil temperature and percentage of soil moisture are subject to considerable error. In the matter of ground and soil temperature, it was impossible to take these at exactly the same hour of the day. The difficulty of sampling due to the great variation in topography, rockiness, and extreme dryness and hardness of the soil made uniformity in the matter of soil temperature and the collection of soil samples difficult.

For stations no. 2, 3, and 4 the severest conditions exist in July, with both ground and soil temperature running higher in the Digger

pine-oak woodland than in the oak savanna area. The percentage of soil moisture shows a rather uniform decrease throughout the season, with only marked decrease in August at station no. 2 in the oak savanna.

The generally greater severity of conditions at station no. 3 in the Digger pine-oak woodland, as indicated by the available data, does not seem consistent with the distribution of vegetation. The difference in altitude between stations no. 2, oak savanna, and no. 3, Digger pine-oak woodland, is a matter of about 250 feet and a distance of some 4 miles. *P. sabiniana*, however, is not found in the area of station no. 2, whereas in the area represented by station no. 3 it is a conspicuous and dominant feature of the vegetation. The difference in environmental conditions between stations no. 3 and no. 4 as indicated by these data represents a change toward a much more mesophytic condition, with dominance of a conifer forest just above station no. 4.

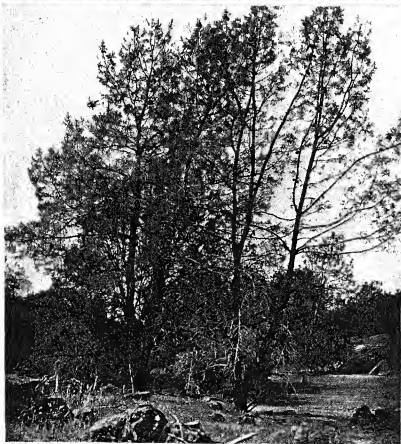
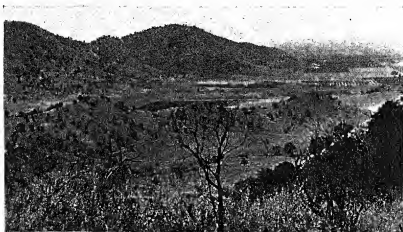
SUCCESSION

COOPER (3) believes that the chaparral formerly occupied a much greater area on the lower slopes of the Sierra Nevada. From relict evidence in the Sacramento Valley he thinks there is a possibility that it may in times past not only have controlled a much larger portion of the foothill area here discussed, but also may have been dominant in the area now controlled by the valley grassland, or would be controlled by the grassland were it not for the interference of agricultural development.

The vegetation of the area has undoubtedly been greatly modified by a long period of human occupation. There were several large Indian rancherias between the Kings and the San Joaquin rivers throughout the Digger pine-oak woodland at the time of the first white settlements in 1850. This elevation was one of the main centers of Indian habitation and had long been occupied,² as evidenced by the numerous rock pot holes which were used for grinding the acorns of *Q. douglasii* and the seeds of *P. sabiniana*. The Indians, according to the early white settlers, made a practice of setting fire to the grass on the foothills with the idea of keeping down the development of brush and maintaining an open parklike woodland.

² Verbal information from L. A. Winchell, a pioneer resident.

With the coming of placer miners and the more permanent settlement of the early stockmen this burning practice was continued. Many stockmen today contend for the practice of annual burning



FIGS. 10, 11.—Fig. 10, oak savanna of frontal foothills; *Quercus douglasii* and *Q. wislizenii* with *Avena* spp. in foreground. Fig. 11, Digger pine-oak woodland; *Pinus sabiniana* in foreground with *Q. douglasii* in rear. Dichotomous branching and open broomlike tops are characteristic of former.

to restrict development of brush, as against the Forest Service policy of fire control.

With more permanent settlement of the foothill area, agricultural development has constantly encroached on the native vegetation where topography has permitted it. Clearing for grain fields, orchards, and to a small extent for vineyards has proceeded steadily. This, combined with road building and the great development of the main timber belt above as a recreation area, has materially increased the fire hazard with its resulting effect on vegetation.

The question of succession in the foothill area is a matter of considerable economic importance. The chief possibilities of the area are in its usefulness for grazing, watershed, and recreational purposes. The secondary successions which are resulting from fire become therefore of importance. The Bureau of Forestry (12), in its study of cover type in relation to fire control in the forest of northern California, has found that the chaparral and woodlands in relation to other vegetation types rank high in length of fire season, number of incendiary fires, rapidity of burning, acreage burned over, and cost of control. The frequent destruction of the plant cover by fire accelerates erosion and decreases watershed value, while new plant successions interfere with the other possibilities of usefulness of the foothill area.

TYPES OF SUCCESSION

Owing to the causes just discussed, the native vegetation has been greatly changed by human occupation, and areas of primary succession are limited. Successions in all cases, because of the xeric nature of the habitat, appear to be greatly telescoped with chaparral, shrubs appearing soon after or at the same time as the pioneers. In limited areas primary xeric successions may be seen as follows.

I. ROCK FACES AND CREVICES.—On the granitic and basaltic rocks, lichens of the crustose type are common. As crevice plants, *Gymnogramme triangularis*, *Pellaea ornithopus*, *Selaginella bigelovii*, *Cotyledon laxa*, *Panicum hirticaule*, *Nemophila aurita*, and *Phacelia californica* are common.

II. WEATHERED ROCKY HILL TOPS—BASALTIC CAPS OF TABLE MOUNTAINS.—Weathering here has produced small accumulations of soil and conditions are somewhat more favorable than those in I. Spe-

cies previously listed, with isolated specimens of *Quercus douglasii*, *Q. wislizenii*, and *Pinus sabiniana*, make up the bulk of the vegetation. Where there has been disintegration by weathering a condition approaching the chaparral is attained but with less density of stand. In numerous cases associations of *Rhamnus californica* and *Ceanothus cuneatus* prevail. Basaltic crevices in spring produce regular patterns of flowers when covered with societies of *Baeria chrysotoma*.

III. TALUS AND HILLSIDE SLOPES.—Talus slopes are limited to the sides of the table mountains where the disintegrating lava caps form talus slopes of small area. These terminate on gravelly slopes resulting from gravels and conglomerates underlying the basalt and offer a poor foothold for plants. On south- and west-facing slopes the vegetation is very scanty, but even in these extremely xerophytic conditions *P. sabiniana* occurs. Isolated specimens may be found even to the tops of the tables. With it as individuals or restricted associations are *Q. douglasii*, *Q. wislizenii*, *Rhus diversiloba*, *Lupinus albifrons*, *Senecio douglasii*, *Ceanothus cuneatus*, *Aesculus californica*, and *Sambucus velutina*. On north slopes the vegetation is comparable with the chaparral of higher elevations, with *Q. wislizenii* particularly prominent.

Because of the very nature of the climatic situation, mesic primary successions are extremely limited. The only perennial streams in the foothill area are the two main rivers. Numerous ephemeral streams run for a short time during the winter, but owing to the extreme drought of the last two seasons several of these have been dry. Occasional springs cause some standing water on rock basins during the spring.

SECONDARY SUCCESSION

Secondary succession is initiated by two primary causes: agricultural usage and fire or a combination of both. With the exception of the "table mountains," the topography is rough with low hills rising to a height of 2500-3500 feet. Although there has been considerable decomposition into shallow soils, in many places the granite is exposed in large blocks and patches. Many small valleys have been put under cultivation. In clearing the land fires have been allowed to run to the tops of the hills or to some natural fire break.

In abandoned fields succession may be observed and is much telescoped. In the oak savanna *Quercus douglasii* may be seen invading open grassland, as well as *Ceanothus cuneatus* which develops into dense stands of rather limited area. In especially xerophytic situations *Lotus scoparius* is a pioneer and is succeeded by *Ceanothus*. Much the same succession occurs at somewhat higher elevations, except that with *C. cuneatus* are associated *Arctostaphylos mariposa* (although it does not reach a large size), *Rhus trilobata*, *R. diversiloba*, and *Rhamnus crocea*.

Where the burning has been so severe that practically the whole woodland formation has been destroyed, *Eriodictyon californicum* is the principal pioneer, to be followed by the shrubs of the chaparral.

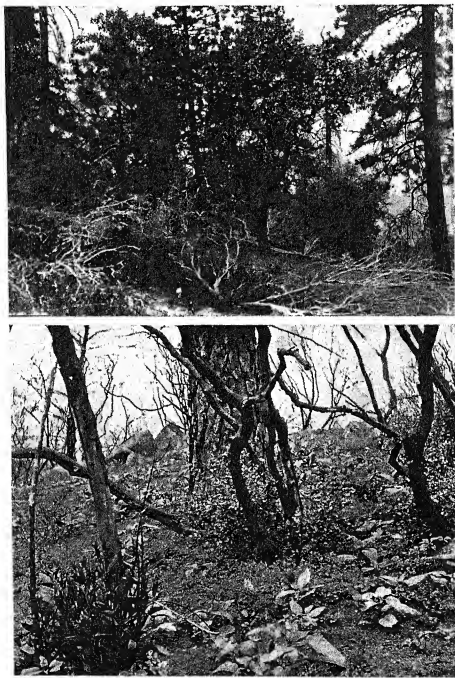
Where the chaparral itself is destroyed by fire, several of the principal woody elements are able to reproduce themselves promptly by root sprouts. In a permanent quadrat on the Auberry road at an elevation of about 3100 feet, which was burned in July, 1930, crown sprouts of *Quercus wislizenii*, *Aesculus californica*, and *Umbellularia californica* were 15-30 inches high on May 1, 1931. Especially conspicuous features of reproduction in this burn are the large number of seedlings of *Eriodictyon californicum* and the production of *Fremontia californica* from running roots as well as basal sprouts. In numerous cases sprouts from roots were observed as far as 15 feet from the parent plant. They were produced in great numbers and the young shoots were making vigorous growth. In this burn all specimens of *P. sabiniana* and *Arctostaphylos* were killed by the fire of July, 1930. *Quercus*, *Umbellularia*, *Fremontia*, and *Aesculus* are rapidly and successfully renewing themselves by root sprouts, whereas *Arctostaphylos* seedlings were numerous.

The summer of 1931 was extremely severe. The total precipitation was below normal, while for four of the six months from April to September inclusive the mean temperature, according to records at Fresno, exceeded the average mean of the last 34 years.

When this burn was examined in October, 1931, the effect of the desiccating summer upon chaparral plants was found to have been the destruction of practically all the *Arctostaphylos* seedlings, while those forms reproducing by root sprouts, although reduced in numbers and vigor, were maintaining themselves.

The development of seedlings is an interesting feature of this

burn. In the spring of 1931 a large number of herbaceous species, a list of which is attached, invaded the burn with *Montia perfoliata* especially prominent. As the season advanced other herbaceous and



FIGS. 12, 13.—Fig. 12, contact between chaparral and yellow pine forest; atmometer station no. 4. Fig. 13, reproduction in chaparral about 9 months after heavy fire. All *Pinus sabiniana* and *Arctostaphylos mariposa* killed; *Quercus wislizenii* and *Umbellularia californica* rapidly reproducing by root sprouts. Numerous seedlings of *Eriodictyon californicum* in foreground.

woody forms became more prominent, especially the slower-starting woody perennials such as *Eriodictyon californicum*. This species is always a feature of open roadsides and other situations of intense light and heat. To determine the number of seedlings, an accurate count was made of a 5-foot quadrat. There were 21 specimens of *Montia perfoliata*, 76 of *Eriodictyon californicum*, 3 of *Mentzelia dispersa*, and 126 of *Ceanothus divaricatus*. The presence of this last species is interesting as it usually occurs in the upper limits of the chaparral and in this area there are no large stands of mature individuals.

Several local showers had caused a late-season development of grasses and herbs in this burn. The following is a list that could be identified in the permanent quadrat up to June 1, 1931.

Grasses and herbs invading permanent quadrat

Amaranthus graecisans	Gilia multicaulis
Anthemis cotula	Lotus scoparius
Arabis holboellii var. arcuta	Lupinus benthami
Avena fatua	Marrubium vulgare
A. barbata	Matricaria suaveolens
Brodiaea capitata	Mentzelia dispersa
Bromus hordeaceus	Mimulus torreyi
B. rubens	Montia perfoliata
Chenopodium carinatum	Plagiobothrys nothofulvus
Cirsium californicum	Poa annua
Collinsia tinctoria	Phacelia californica
Cryptantha flaccida	Polygonum aviculare
Delphinium decorum	Pterostegia drymarioides
Dendromecon rigida	Salvia columbariae
Erodium cicutarium	Scutellaria angustifolia
Eschscholtzia caespitosa	Silene antirrhina
Festuca megalura	Solanum xanthii
Galium nuttallii	Streptanthus diversifolius
Gilia giliioides	

Upon other burns in the same vegetative area and under the same conditions of topography where fire occurred 5-10 years previously, the succession has moved forward. The area is covered with a

chaparral growth, 4-8 feet high and considerably tangled. *Quercus wislizenii* composes about 40 per cent of the vegetation, *Eriodictyon* 30 per cent, *Ceanothus cuneatus* 20 per cent, with scattered specimens of other chaparral shrubs making up the remainder. The succession following fire at the edge of the forest area is a chaparral formation which includes a mixture of a wide variety of woody plants, of which *Q. wislizenii*, *Umbellularia californica*, and *Pinus sabiniana* attain tree form; whereas in the oak savanna, and par-



FIG. 14.—Reproduction of *Pinus sabiniana* and *Ceanothus cuneatus* after an old fire

ticularly in the Digger pine-oak woodland, the succession form in the pioneer stages tends to be an almost pure stand of *Ceanothus cuneatus*.

The relationship between the chaparral and the line of contact with the yellow pine forest can be seen just above this burned area. The point of contact is approximately 4000 feet in elevation and there are definite indications that the chaparral has pushed back the yellow pine for a noticeable distance. Typical chaparral plants, as *Quercus wislizenii*, *Arctostaphylos mariposa*, and *Pinus sabiniana*, invade for some distance the yellow pine area. In view of the observed susceptibility of *P. sabiniana* to fire, and the youth of the trees, as evidenced by borings, it would seem that this invasion is a succession resulting from fire along the edges of the conifer forest.

On west and south exposures, where the slopes are steep, this chaparral formation tends to persist as a climax, with the arboreal elements, as *Quercus* spp. and *Pinus sabiniana*, tending to crowd out the shrubby specimens to form a climax woodland or sclerophyll forest in contact with a climax yellow pine forest, unless the succession is again checked by fire.

The relation of *Pinus sabiniana* to these successions is significant. It occurs throughout the upper portion of the woodland area here designated as the Digger pine-oak woodland. It is an integral part of the chaparral, and as such is invading the yellow pine forest for short distances as part of the present succession. It does not occur, however, below certain clearly marked levels, usually about 600 feet in elevation, the area below being an oak savanna. This definite zonation seems to be the result of two sets of factors. The first is climatic and the second is involved in its successional relationships. It is a tree particularly adapted to the xerophytic conditions of the foothills; and there is apparently no reason why it should not develop on the first foothills now dominated by the oak savanna, except for the greater hazard of and its particular susceptibility to fire. On its upper limits as part of the chaparral formation it may invade the yellow pine forest. If fire is kept out, it, with the chaparral, will be succeeded by the yellow pine forest.

The peculiar gap in the distribution of *Pinus sabiniana* between the Kings and White rivers can possibly be explained entirely on the basis of fire. In all the burned areas examined this species seems to have suffered most heavily. The vacant area may be accounted for on the supposition that fire runs to natural barriers, such as the rivers, and that it has not as yet succeeded in reestablishing itself. This process is now taking place, for although the Kings River is a difficult barrier, it has again crossed this stream and formed excellent stands on river benches and hill slopes some half mile south of the river.

Summary

1. *Pinus sabiniana*, a pine restricted to California but widely distributed on the interior coast ranges and the lower western slopes of the Sierra Nevada, except for a break between the Kings and White rivers, was studied in its ecological relationships.

2. The woodland formation of which *P. sabiniana* is a part is in contact at its lower limits, at an elevation of 600 feet, with an oak savanna which forms a narrow belt above the grassland of the Great Valley and lower foothills.

3. The area chosen for intensive study is bounded by the Kings and San Joaquin rivers and extends from the floor of the valley to about 4000 feet elevation.

4. Quantitative data were obtained by the use of atmometers at four stations typical of the main vegetative zones, above, below, and within the zones of distribution of this species. Other data were gathered upon precipitation, temperature, ground and soil temperature, and soil water content.

5. In its lower zone of distribution *P. sabiniana* is associated with *Quercus douglasii* and *Q. wislizenii*; in its higher levels it is found with chaparral shrubs and trees such as *Ceanothus cuneatus*, *Arctostaphylos mariposa*, *Fremontia californica*, *Quercus wislizenii*, and *Umbellularia californica*. At its upper limits it grades into hard chaparral, the result of fire or possibly a climax on steep and rocky western and southern slopes.

6. As part of this chaparral it is invading the yellow pine forest on its upper limits but is not moving downward into the oak savanna.

7. Studies of burns, both new and old, show that *P. sabiniana* is easily killed by fire. *Quercus*, *Umbellularia*, *Aesculus*, and *Fremontia*, prominent associates in the chaparral, are reproducing by root sprouts, but *P. sabiniana* must start anew from seed.

8. The data available on environmental factors, as well as a study of succession, indicate that distribution is controlled by its adaptability to the xerophytic conditions of the foothills. It is prevented from intruding into the lower elevations by the greater frequency of fires in the oak savanna, and its upper limits are determined by its relation to the chaparral of which it is a part.

9. Where the chaparral is invading the yellow pine forest as a succession following fire, *P. sabiniana* appears as a part of that succession. If no further fires occur the yellow pine will reestablish itself.

10. The gap in the distribution of *P. sabiniana* between the Kings and the White rivers can possibly be explained entirely upon the relation of this pine to fire, as it has successfully crossed the Kings River, a very formidable barrier, and is slowly advancing southward.

The writer wishes to acknowledge the assistance received from Dr. HENRY C. COWLES in carrying out this work. Thanks are also due to Dr. GEORGE D. FULLER for advice and for the use of instruments, to Mr. W. S. BALLARD for help in maintaining atmometers, and to Mr. C. H. QUIBELL for identifying herbaceous species in the burned areas.

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CYTOLOGY OF ANTENNARIA¹

I. NORMAL SPECIES

G. LEDYARD STEBBINS, JR.

(WITH THIRTY-TWO FIGURES)

Introduction

The phenomenon of apomixis in the higher plants has attracted the attention of cytologists ever since its discovery by experimental methods in the middle of the last century (BRAUN 5). The work of JUEL (24) on the European species of *Antennaria*, however, was the first attempt at a comparison of the cytology of closely related sexual and parthenogenetic species; and showed how, in the entirely parthenogenetic *A. alpina*, the reduction divisions in the pistillate flowers are completely omitted. The results of other workers in many other genera, as summed up by ROSENBERG (35), have demonstrated transitions between the presence and the complete absence of the reduction division in apomictic plants, making the further study of the more numerous American species of *Antennaria* seem highly desirable.

That the genus *Antennaria* in North America constitutes a large, polymorphic complex of species was first recognized by GREENE (15, 16) and FERNALD (11). They showed that instead of a single, very variable species, there exist in the northeastern United States eleven more or less closely related species. Moreover, a great number of species from other parts of North America have been described, so that more than 100 species from North America are now represented in the Gray Herbarium.

That many of the species in the eastern United States reproduce parthenogenetically was recognized by GREENE (17), and was demonstrated cytologically for four species by LEAVITT and SPALDING. Their brief preliminary note (28) was never followed by a detailed account of their results.

The present study aims to continue the work of LEAVITT and SPALDING, and to compare the sexual and parthenogenetic species

¹ Contribution from the Laboratory of Plant Morphology, Harvard University.

found in the eastern United States, particularly as regards their reduction division and the development of the gametes in both sexes, in an effort to discover the cause of the widespread parthenogenesis in the genus.

Material and methods

The material of the species studied was collected during the springs of 1928, 1929, and 1930. Nine of the ten species were found in the vicinity of Cambridge, Mass., while *A. solitaria* Rydb. was collected during a trip to Washington, D.C.

The buds were collected from one to two weeks before anthesis; and owing to the great number of heads in an inflorescence, and of flowers in a single head, there was no difficulty in obtaining all the necessary stages of development from the same colony at one time. Herbarium specimens were made from each colony at a later stage, when the plants were mature, and were carefully compared with authentic material in the Gray Herbarium.

The buds were fixed in Carnoy's fluid with the aid of a vacuum pump, and after 24 hours were washed in 95 per cent alcohol. They were then treated with dilute hydrofluoric acid in 70 per cent alcohol for 12 hours, to remove the mineral matter in the hard receptacles and pappus hairs, and imbedded in nitrocellulose according to JEFFREY'S (23) method. The staminate heads were imbedded whole, but in the pistillate flowers a larger number of the necessary longitudinal sections was secured by removing them from the receptacles and spreading them out separately on cards. Sections were cut 8-12 μ in thickness for the reduction divisions and embryo sac development, while 15-20 μ was found to be a more satisfactory thickness for the older embryo sacs and young embryos. The sections were stained with Haidenhain's iron-alum haematoxylin, and those of the pistillate material counterstained with eosin.

Observations and drawings were made with a Zeiss microscope, a 1.5 mm. apochromatic objective, and a 10 \times eyepiece, except in the case of the embryo sacs, where the 3 mm. apochromatic objective with the 10 \times eyepiece gave sufficient magnification. The drawings were made with the aid of a camera lucida, and many of them were enlarged 1.4 and 2 diameters with the aid of a pantograph. The resulting magnifications are given with the descriptions of the plates.

Observations

The genus *Antennaria* is distinguished from its near relatives in the Compositae by its dioecism. The staminate flowers have only the outer integuments of the achene and a short, abortive style and stigma, while the pistillate flowers have only the rudiments of filaments. The staminate and pistillate plants are further distinguished from each other by a number of secondary sex characters, as described by JUEL (24).

MICROSPOROGENESIS IN *A. PLANTAGINIFOLIA* AND *A. NEGLECTA*

Since micro- and megasporogenesis in *A. plantaginifolia* and *A. neglecta* are essentially the same, these two species will be described together. *A. plantaginifolia*: no. 253, from Turkey Hill, Arlington, Mass.; no. 260, grassy roadside, South Braintree; no. 486, Canton Junction; *A. neglecta*: nos. 481 and 483, Berlin, Mass.; no. 503, Wilmington, Mass.

The material is not very favorable for the study of the early prophase, and both for this reason and also because it has no connection with the problem of apomixis, the study of these stages was omitted. During diakinesis the chromosomes appear as short rods, most of them paired end to end, although O- and X-shaped pairs are frequent (fig. 1). In later diakinesis fourteen pairs of bivalent chromosomes can frequently be counted with certainty, while unpaired univalents have never been seen in either species at this stage. In the heterotypic metaphase, the bivalent pairs arrange themselves regularly at the equator of the bipolar spindle (fig. 2), and in the polar view the number fourteen can easily be counted (figs. 3, 4). In favorable cell plates, variations in shape and size of cross-sections of the chromosomes can be observed, and it is seen that there are seven sets of two similar pairs of bivalents, the members of each set of two usually being associated or close to each other on the plate. Thus, in figure 2, there are in the upper left-hand corner of the cell plate two small, round bivalents, with two large, oblong ones just below them. Near the lower left-hand corner are two bivalent pairs very closely associated, apparently forming a quadrivalent group, while

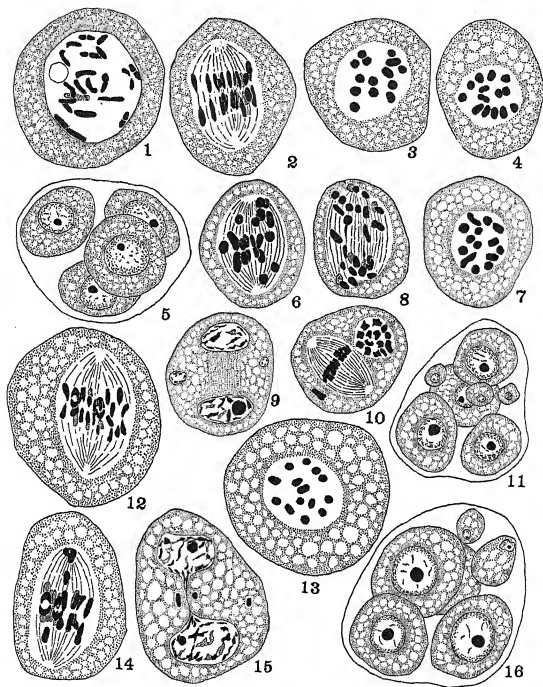
there is a line of four bivalent pairs along the bottom of the cell plate, of which the two pairs at the left are similar in size and perceptibly smaller than the two right-hand ones. Similar arrangements may be seen in figure 3.

A more detailed study of the individuality of the chromosomes in these species will be made in the future, and not until then can the presence or absence of unequal, X-Y pairs of sex chromosomes be definitely established.

The bivalent pairs separate regularly for the early anaphase, although two of them frequently start to separate slightly before the other twelve. They proceed regularly to the poles, and in the heterotypic anaphase fourteen chromosomes can be clearly counted at either pole.

At the end of the anaphase the chromosomes become closely clumped together, and a nuclear membrane forms around them. The interkinesis is long, and the chromosomes lose their identity, although the nucleolus does not generally form. The homoeotypic division is also regular, and in favorable cell plates the fourteen chromosomes can again be counted. The four nuclei formed by this division form a normal tetrad of four microspores, which develop into densely protoplasmic, 2-nucleate pollen grains. All the grains are of the same size, and the pollen is 100 per cent perfect.

Abnormal deviations from this regularity of microsporogenesis were found in one collection of *A. plantaginifolia* (no. 491, from Horn Pond Hill, Winchester, Mass.). This form, although with the leaf, stolon, and inflorescence characters of typical, staminate *A. plantaginifolia*, differed from the typical forms in its pappus hairs with narrower, more sharply serrate tips, thus approaching the shape of the pappus hairs in the pistillate flowers. In this form, lagging chromosomes occasionally occur in the heterotypic division, which are left out of the daughter nuclei at the heterotypic telophase and form small extra nuclei. Thus polysporic "tetrads" are formed. These are about 15 per cent of the total number of tetrads. The aberrant character of the pappus hairs in this form suggests the presence of heterozygosity, which would account for the irregularities observed.



FIGS. 1-16.—Fig. 1, *A. plantaginifolia*, microsporogenesis, diakinesis. Fig. 2, heterotypic metaphase. Fig. 3, same, polar view. Fig. 4, *A. neglecta*, heterotypic metaphase, polar view. Fig. 5, tetrad. Fig. 6, *A. neglecta* × *plantaginifolia*, heterotypic metaphase. Fig. 7, same, polar view. Fig. 8, heterotypic anaphase. Fig. 9, interkinesis. Fig. 10, homeotypic metaphase. Fig. 11, polysporic heptad. Fig. 12, *A. solitaria*, heterotypic metaphase. Fig. 13, same, polar view. Fig. 14, same, showing irregularities. Fig. 15, interkinesis. Fig. 16, polysporic hexad. ×2400.

MEGASPOROGENESIS IN *A. PLANTAGINIFOLIA*
AND *A. NEGLECTA*

The following pistillate plants were studied. *A. plantaginifolia*: no. 258, from roadside, South Braintree, Mass.; no. 492, dry wooded hillside, Belmont, Mass.; nos. 506 and 508, grassy hillside, Forest Hills, Boston; no. 749, dry field, Ballston, Virginia. *A. neglecta*: no. 484, dry hillside, Berlin, Mass.; no. 264, grassy knoll, near Braintree, Mass.; no. 517, dry field, Lexington, Mass.

Diakinesis in the megaspore mother cell is similar to that in the microspore mother cell, although the nucleus is in the former somewhat larger, and the chromosomes are more easily counted. The heterotypic division proceeds regularly (figs. 17, 18), and in one or two side views of metaphases and anaphases the haploid number of fourteen chromosomes could be made out. The heterotypic spindle is always oriented along the longitudinal axis of the ovule. At the heterotypic telophase a cell membrane forms, which persists. During interkinesis, the chromosomes lose their identity and a nucleolus forms in each nucleus (fig. 19). The homoeotypic division is regular (fig. 20), and forms a tetrad of megaspore cells with definite cell walls between them (fig. 21).

During both the heterotypic and the homoeotypic divisions there is a definite dense area of cytoplasm or perinuclear zone surrounding the nuclear structures, which is more distinct than that seen in the similar stages of pollen development.

Of the four cells in the megaspore tetrad, the chalazal one soon expands at the expense of the other three, which degenerate. It grows rapidly, and soon pushes through the nucellar cells, which degenerate. Three equational divisions occur, of which the first is shown in figure 28, resulting in a long, narrow, 8-celled embryo sac (fig. 29). This embryo sac expands chiefly at the micropylar end, so that it becomes flask-shaped (fig. 30). The polar nuclei fuse during the early 8-celled stage. The three antipodal nuclei divide repeatedly, forming cell membranes between them, so that at maturity there is an antipodal tissue of 15-20 cells.

Embryo development occurs after fertilization in the usual manner, and in both species pollen tubes were regularly seen in the older material.

Experiments to test the presence or absence of apomixis were performed by the following methods: 1. The styles and stigmas were removed from the unopened buds by cutting across the heads just above the achenes with a sharp razor blade. 2. The pistillate inflorescences were inclosed, before the oldest flowers had opened, in a paraffin paper bag which was removed three or four weeks later.

In both *A. plantaginifolia* and *A. neglecta*, these experiments resulted in complete failure of the achenes to develop, although they were repeated on a number of plants, and untreated heads in the colonies showed a good development of achenes. There is, therefore, no evidence whatever that apomixis exists in either of these two species.

A. NEGLECTA × *PLANTAGINIFOLIA*.—There are a number of specimens of this hybrid in the Gray Herbarium, while the writer has found that pistillate plants which are evidently intermediate between the two species are not uncommon, although the staminate plants are rarer.

As a guide to the systematic characters of the various hybrid plants studied, a brief summary of the differences between *A. neglecta* and *A. plantaginifolia* is here given.

A. neglecta: Rosette leaves 1.5–5 cm. long by 0.5–1 cm. broad, spatulate, with one prominent midrib; stolons long, procumbent; inflorescence of staminate plants subcapitate, with 2–4 heads; staminate corolla 3.2–4 mm. long; staminate pappus hairs with slightly dilated, crenate tips (fig. 31 *a*); inflorescence of pistillate plants subcapitate, becoming racemose in age, with 2–5 heads; pistillate flowers with corollas 4.5–5.5 mm. long, and with slender, slightly serrate pappus hairs (fig. 31 *b*).

A. plantaginifolia: Rosette leaves 2.5–8 cm. by 0.7–4 cm., ovate-ellipsoid, 3-ribbed, the ribs conspicuous; stolons short, leafy, assurgent; inflorescence of staminate plants subcapitate to corymbose, with 3–12 heads; staminate corollas 2.6–3.3 mm. long; the pappus hairs with much dilated tips and sharply serrate below (fig. 31 *c*); pistillate inflorescence corymbose, heads 5–15; pistillate corollas 3–4 mm. long, the pappus hairs stouter, with long, sharp teeth (fig. 31 *d*).

The hybrids include forms intermediate in these characters.

HYBRID STAMINATE PLANTS

The following staminate plants were studied: no. 478, meadow, Winchester. Leaves $2.5-3.5 \times 0.7-1$ cm., spatulate to narrowly elliptic with a single prominent midrib, but more veined than in *A. neglecta*; stolons procumbent; inflorescence subcapitate, with 5-6 heads; corolla 3.3 mm.; pappus hairs intermediate. The chromosomes all pair at diakinesis. In the heterotypic metaphase, most of the cells show fourteen pairs of bivalents at the equatorial plate, but frequently there are two to four unpaired chromosomes, which are scattered near the poles. In the heterotypic anaphase these chromosomes, or sometimes the members of a bivalent pair, lag in the center of the spindle and are left out of the nuclei formed at the heterotypic telophase. They form small extra nuclei at interkinesis, but during the homoeotypic division the nuclear membrane disappears from them, and they show as dark staining masses of chromatin in the cytoplasm.

The homoeotypic division proceeds regularly. At the homoeotypic telophase the four usual nuclei are formed, as well as small extra nuclei, formed around the masses of chromatin in the cytoplasm. This results in polysporic "tetrads." These constitute 15 per cent of the total.

No. 753, dry hillside, Braintree. Stolons not developed; inflorescence subcapitate, with 4-5 heads; pappus hairs intermediate. Here were found 2-4 unpaired chromosomes in 50 per cent of the heterotypic metaphases seen. Polysporic tetrads constitute 26 per cent of the total.

No. 752, dry field, Belmont. Leaves $2.5-3.5 \times 0.7-1$ cm., elliptic, pointed, the larger 3-ribbed; stolons becoming assurgent; heads 4-5, in a subcapitate inflorescence; pappus hairs intermediate. In diakinesis the members of one or two pairs of chromosomes are frequently rather loosely associated. In the heterotypic metaphase there are always from 2 to 10 chromosomes lying scattered on the spindle. Frequently two similar chromosomes, obviously the members of a bivalent association which have not paired properly, are seen lying close to each other near a pole of the spindle. Two such chromosomes are seen near the upper pole in figure 6, which shows a case of ex-

treme irregularity for this plant. The haploid number of fourteen can sometimes be counted at the heterotypic metaphase, as in figure 7. The heterotypic anaphase is frequently very irregular, as in figure 8, and there are not uncommonly two to three extra nuclei at interkinesis (fig. 9). The homoeotypic division proceeds regularly, except for the masses of chromatin representing chromosomes left out at the heterotypic telophase (fig. 10). There are sometimes as many as three extra cells in the "tetrads," and polysporic "tetrads" constitute 44 per cent of the total.

No. 490, rocky hillside in scrub, Winchester. Leaves $3.5 \times 0.7-1$ cm., elliptic, 3-ribbed; stolons short, assurgent; heads 4-5, in a capitate inflorescence; corolla 2.7 mm.; pappus hairs as in *A. plantaginifolia*. In this form lagging univalents are rare in the heterotypic metaphase and anaphase, and there is only 4 per cent of polysporic "tetrads."

It is thus seen that nos. 478 and 490, which resemble more closely *A. neglecta* and *A. plantaginifolia* respectively, show fewer abnormalities in the reduction division than nos. 752 and 753, which are almost exactly intermediate between the two species.

HYBRID PISTILLATE PLANTS

The following pistillate plants were studied:

No. 514, dry field, Bellevue Hill, Boston. Leaves $2.5-4.5 \times 0.7-1$ cm., somewhat elliptic, much veined; stolons becoming assurgent; heads 5-7, in a dense, corymbose inflorescence; corolla 3.8 mm.; pappus hairs with sharp teeth. Mature embryo sacs showed no abnormalities, although there were many degenerate achenes and many flowers in which the stigma was already withered, although the egg had not yet developed. Castration experiments resulted in the apparent parthenocarpic development of a few achenes, but no embryos could be found in any of the castrated or bagged material. The unbagged heads in the same colony showed a fertility of only 0-38 per cent, but some achenes containing embryos were found in all but one of the heads.

No. 495, dry field, golf course, Belmont. Leaves $2-2.5 \times 0.8-1.1$ cm., the larger 3-ribbed; stolons becoming assurgent; heads 7; inflorescence corymbose; corolla 4.5 mm.; pappus hairs intermediate. Bag-

ging experiments showed no development of the embryos, while unbagged heads in the same plants produced very few good achenes.

No. 754, dry rocky knoll, Braintree. Leaves 3.5-4.5×0.8-1.1 cm., narrowly elliptic, 1-ribbed; stolons assurgent; heads 3-6, in a dense, corymbose inflorescence; corolla 4.2 mm.; pappus hairs intermediate. Here again bagged inflorescences produced no good achenes, while unbagged heads in the same plant produced a few good, plump achenes.

No. 519, dry field, Lexington. Leaves 2-5×0.9-1.3 cm., narrowly obovate, not strongly veined; stolons mostly procumbent; inflorescence densely corymbose; pappus hairs intermediate. This was the only pistillate form in which the reduction division was observed. At diakinesis there are pairs of bivalent and unpaired univalent chromosomes. Figure 22 shows eleven pairs of bivalents and six univalents. In the heterotypic metaphase there are generally from one to four chromosomes lying scattered at the center of the spindle.

In the heterotypic anaphase there is always some lagging, while figure 25 shows one large and one small extra nucleus at interkinesis. During the homoeotypic division the membranes disappear from the extra nuclei, and they appear as bits of chromatin in the cytoplasm. The homoeotypic division is usually regular. Most of the tetrads have one or two small extra nuclei (fig. 27). The development of the embryo sac is normal up to the young 8-celled stage, the oldest stage observed.

No. 524, clearing in pine woods, Monponsett, Halifax, Mass. Leaves 3-5×0.9-1.5 cm., obovate, rounded; stolons procumbent; inflorescence densely corymbose; heads 8; corolla 3.2 mm.; pappus hairs approaching *A. neglecta*. The youngest stages obtained were tetrads, of which a number contain extra nuclei. The normal development of the embryo sac, illustrated in figures 28-30, occurs in most cases.

One notable exception to this normal development of the embryo sac is illustrated in figure 32. Here a 4-celled embryo sac is degenerating, and another, more densely protoplasmic embryo sac is pushing its way between the chalazal and micropylar ends of the original one, breaking it in two. Only three nuclei were seen in this adventive embryo sac, but its chalazal end was cut off by the knife, and since

the section was not one of a series, this end was lost. Repeated attempts to discover other examples of this phenomenon at different stages, both in this and in other examples of *A. neglecta* \times *plantaginifolia*, have failed. It is probable that this adventive embryo sac originated from one of the nucellar cells, although most of them have apparently degenerated, or more likely from one of the cells of the integument. In either case the nuclei of this embryo sac would have the unreduced number of 28 chromosomes.

No castration or bagging experiments were performed on this plant. The negative results of such experiments on all the forms of *A. neglecta* \times *plantaginifolia* on which they were performed indicate that this phenomenon is probably of very rare occurrence.

MICROSPOROGENESIS IN *A. SOLITARIA*

A. solitaria Rydb., staminate material from Riggs's Mill, Prince George County, Maryland. This species has considerably larger flowering heads than *A. neglecta* or *A. plantaginifolia*, and the anthers and pollen mother cells are correspondingly larger.

At the heterotypic metaphase the chromosomes, although of about the same size as those of *A. neglecta* and *A. plantaginifolia*, are usually more widely separated, and their individuality can more easily be made out. It is seen in figure 12 that they vary considerably in size and shape, and that two pairs of similar size and shape can frequently be discerned. The haploid number of fourteen is easily counted in the polar view of the metaphase cell plate.

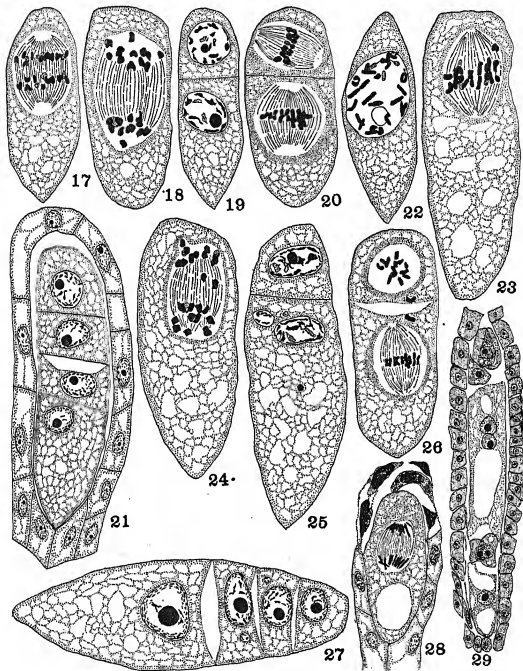
The heterotypic and homoeotypic divisions generally proceed regularly, and a normal tetrad of four microspores is formed. However, unpaired, lagging chromosomes were observed in a very few heterotypic metaphases and anaphases (fig. 14). Figure 15 shows a heterotypic telophase in which a lagging chromosome has been stretched out to form a narrow bridge of chromatin between the two daughter cells. Figure 16 shows a hexad with two microcytes. Such irregularities are rare in *A. solitaria*, and there is only 4 per cent of abnormal tetrads. The pollen is about 98 per cent perfect.

No pistillate material of this species could be obtained.

Discussion

CHROMOSOME NUMBER.—The chromosome number of the three sexually reproducing species of *Antennaria* in the northeastern

United States is here established as $n=14$. This corresponds with the highest number given by JUEL (24) for the sexual *A. dioica*



FIGS. 17-29.—Fig. 17, *A. plantaginifolia*, megasporogenesis, heterotypic metaphase. Fig. 18, *A. neglecta*, heterotypic metaphase. Fig. 19, *A. plantaginifolia*, interkinesis. Fig. 20, *A. neglecta*, homoeotypic metaphase. Fig. 21, *A. plantaginifolia*, tetrad. Fig. 22, *A. neglecta* × *plantaginifolia*, megasporogenesis, diakinesis. Fig. 23, heterotypic metaphase. Fig. 24, heterotypic anaphase. Fig. 25, interkinesis. Fig. 26, homoeotypic metaphase. Fig. 27, tetrad. Fig. 28, anaphase, first embryo sac division. Fig. 29, young 8-celled embryo sac. Figs. 17-27, ×1800; fig. 28, ×1200; fig. 29, ×600.

Gaertn., which he considered to have between 12 and 14 as the haploid number of chromosomes. From the present study it seems most likely that $n=14$ is the correct number for *A. dioica* as well.

Whether this is the fundamental number for the genus is made doubtful by the existence of the groups of similar pairs of bivalents noticed in each species. "Secondary association" was first noted by ISHIKAWA (22) in the octoploid *Dahlia variabilis*, where at the heterotypic metaphase the bivalent pairs are grouped more or less definitely into quadrivalent associations. LAWRENCE (27) confirmed these observations on *D. variabilis*, and concluded that the species has originated as a hybrid between two tetraploid species. Trivalent groups were noted by BELLING (2) in a triploid *Canna*, while BELLING and BLAKESLEE (3) noted trivalents and quadrivalents of a similar nature in triploid and tetraploid races of *Datura*, and such groups have been frequently observed since then. LONGLEY (29) noted the association of similar bivalent pairs in the tetraploid *Rubus caesius* var. *turkestanicus*, while CRANE and DARLINGTON (7) observed complete secondary association in a tetraploid hybrid between the diploid *Rubus rusticanus* var. *inermis* and the tetraploid *R. thyrsiger*. DARLINGTON (8) found quadrivalents, as well as secondary association, during the heterotypic metaphase in tetraploid species of *Prunus*, while MEURMAN (30) in the 22-ploid *P. lauro-cerasus* observed sexivalent and octivalent associations of chromosomes.

In *Antennaria neglecta*, *A. plantaginifolia*, and *A. solitaria*, not more than one quadrivalent group of chromosomes is found at the heterotypic metaphase, and this was not clearly distinguished at diakinesis, but the presence of more or less close secondary association at the heterotypic metaphase, and the duplication of chromosome types, make it possible that these species are tetraploids, having originated at some remote period from species with seven as the haploid chromosome number. It is significant that the group of species studied, with their basal rosettes, reduced cauline leaves, and stoloniferous habit are distinctly more specialized and thus apparently more highly evolved than *A. carpathica* (Wg.) Bl. and Fing. and its American relatives, which have no prominent basal rosettes, no prostrate leafy stolons, and possess well developed cauline leaves.

It is likely that some species of this latter group, when examined, will be found to have seven chromosomes as the haploid number.

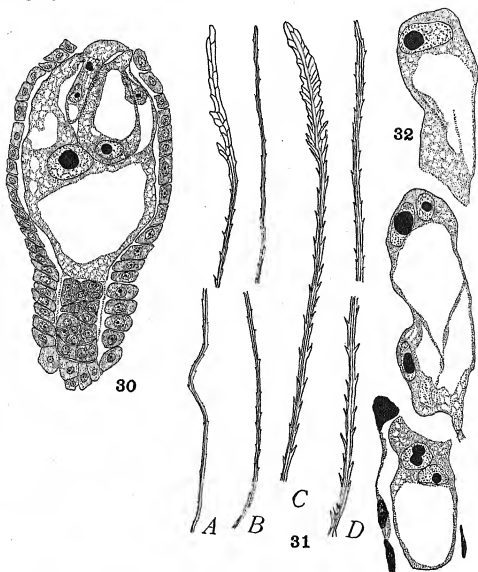
It is notable that *Antennaria* is the only genus yet known of the Compositae in which seven or fourteen is the basic chromosome number (GAISER 13, 14).

HYBRIDIZATION AND CHROMOSOME IRREGULARITIES.—Since the work of ROSENBERG (33) on *Drosera*, the connection of chromosome irregularities with hybridization has been generally recognized, and many workers have since shown the presence of such in known hybrids between species with different chromosome numbers. In hybrids between species having the same number of chromosomes there may be complete homology, and hence complete pairing of the parental chromosomes, with consequent regularity of meiosis, as in hybrids between seven chromosome species of *Fragaria* (ICHIJIMA 21), *Nicotiana langsdorffii* \times *alata* and *N. langsdorffii* \times *sanderæ* (KOSTOFF 26); there may be absolute failure of the chromosomes to pair, as in *Digitalis lutea* \times *lanata* (HAASE-BESSELL 18), and *Raphanus sativus* \times *Brassica oleracea* (KARPECHENKO 25); or there may be all stages intermediate between these extremes. The number of paired chromosomes in such forms may vary greatly among different flowers of the same form, and even among different pollen mother cells of the same anther, and is undoubtedly affected considerably by environmental conditions (BORGSTAM 4, DE MOL 31).

In the megaspore mother cells, chromosome irregularities occur less frequently than in the pollen mother cells, but irregularities similar to those in the megasporocytes of *A. neglecta* \times *plantaginifolia* occur in *Drosera obovata* (ROSENBERG 33), the sterile and parthenocarpic varieties of *Musa* (D'ANGREMOND 1), and the aposporic species of *Hieracium* (ROSENBERG 32, 34), where polysporic tetrads closely resembling those of *A. neglecta* \times *plantaginifolia* are described.

That failure of non-homologous chromosomes to pair is the chief cause of the irregularities in *A. neglecta* \times *plantaginifolia* nevertheless seems evident, since, as pointed out, the most nearly intermediate forms, which should have an equal number of *neglecta* and *plantaginifolia* chromosomes, show less pairing of bivalents than those more closely resembling one or the other parent, in which, if chromosomes are the bearers of hereditary characters, there should (probably as a

result of back crossing) be a preponderance of homologous *neglecta* or *plantaginifolia* chromosomes.



FIGS. 30-32.—Fig. 30, *A. neglecta* \times *plantaginifolia*, mature embryo sac; $\times 600$. Fig. 31, pappus hairs: A, *A. neglecta*, staminate flower; B, pistillate; C, *A. plantaginifolia*, staminate flower; D, pistillate; $\times 30$. Fig. 32, *A. neglecta* \times *plantaginifolia*, aposporic embryo sac. $\times 1200$.

APOSPORY.—The aposporic embryo sac in *A. neglecta* \times *plantaginifolia* no. 524, although a single case, yet merits attention. Aposporic embryo sacs of this type were first described by ROSENBERG (32, 34) in *Hieracium*, and have since been found in *Artemisia nitida* (CHIARUGI 6), *Ochma multiflora* (FRANCINI 12), and *Oxyria digyna* (EDMAN 9).

The existence of this phenomenon in a presumably hybrid form,

in whose probable parents no such phenomenon was observed, leads to the belief that the apospory resulted from hybridization, thus lending support to the theory of ERNST (10) and WINGE (37). Other cases of apomixis resulting from hybridization are, however, rare. STRASBURGER (36) found signs of "apogamy" in *Alchemilla gemmia*, by some investigators considered of hybrid origin, and complete sterility and "apogamy" in *A. trullata*, also of supposed hybrid origin. HARRISON (19) and HARRISON and PEACOCK (20) found four parthenogenetic females in the progeny of the Lepidoptera *Tephrosia bistortata* \times *T. crepuscularia*. In a subsequent paper evidence will be produced to indicate that the regularly apomictic species of *Antennaria* may also be of hybrid origin.

Summary

1. *Antennaria neglecta* Greene and *A. plantaginifolia* (L.) Richards. both have a haploid chromosome number of fourteen, and regular meiosis in both the micro- and megasporocytes.

2. In both species there are seven groups of two similar bivalent pairs.

3. Experiments demonstrated the complete absence of apomixis in both species.

4. In the hybrid *A. neglecta* \times *plantaginifolia*, irregularities of meiosis occur, these being most abundant in forms most nearly intermediate between the two species.

5. In one form of the hybrid a presumably aposporic embryo sac was observed.

6. In *A. solitaria* the haploid chromosome number is fourteen, and the meiosis in the pollen mother cells is mostly regular.

7. The basic chromosome number in *Antennaria* is probably $n = 7$.

8. The chromosome irregularities in *A. neglecta* \times *plantaginifolia* are due chiefly to non-homology of chromosomes, but are probably affected by other causes.

9. The aposporic embryo sac in *A. neglecta* \times *plantaginifolia* is probably the result of hybridization.

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VASCULAR SYSTEM OF YOUNG PLANTS OF *MEDICAGO SATIVA*

CLARA WOLFANGER WINTER

(WITH THIRTY-FOUR FIGURES)

Introduction

The vascular system of a spermatophytic plant, taken in its entirety, forms a conducting channel through root and shoot, being continued into leaves and branches of the shoot by means of prolongations of the main stelar axis of the stem.

The most critical point in the establishment of the continuity of the vascular axis lies in the region of "transition" between the primary root and the primary shoot. The change from the stelar structure of the root, consisting of exarch strands of xylem with phloem on alternate radii, to the stelar structure of the stem, consisting of endarch xylem collaterally arranged with the phloem, presents a situation which has been variously interpreted by anatomists. The trend toward phylogenetic interpretations of anatomical structures has been a stimulus for an increased investigation of this region.

Of the various theories as to the method of transition, a commonly accepted one is that which prescribes definite types of forking, rotation, and fusion of continuous stem-to-root bundles. Three of these types were proposed by VAN TIEGHEM (6) in 1871, and a fourth by SARGANT (4) in 1900. The theory presupposes a primitive homogeneity of the primary vascular structure which is not consonant with the situation as observed in *Medicago sativa* L. In this plant, the stelar structures of the organs of the shoot, that is, stem, branches, and leaves, are homogeneous to one another and to the secondary, not the primary, vascular tissues of the root.

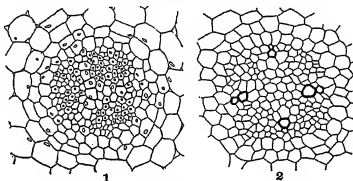
CHAUVEAUD's (1) monumental work on the vascular system presents a recapitulative interpretation of the phenomena observed in the transition region which singularly explains the apparent complications. It is found that seeming anomalies in the primary vascular structure of the seedling of *M. sativa* can be rather nicely explained by CHAUVEAUD's theory.

The bases for this conclusion are observations made by means of serial sections of numerous seedlings and young plants; by means of portions of shoot and root and of entire seedlings cleared in cedar oil or balsam; and by a study of vascular tissues freed from softer imbedding tissues by maceration methods. This paper reports the details of the structure of the vascular system thus studied.

Vascular system

Root

The first evidences of the vascular system appear about 0.25 mm. above the tip of the root. Three, or more rarely four, xylem strands



FIGS. 1, 2.—Transverse sections through root hair region; fig. 1, 2-day-old seedling showing triarchy; fig. 2, 3-day-old seedling showing tetrarchy. $\times 230$.

with alternating phloem groups are differentiated (figs. 1, 2). Lignification and maturation progress centripetally, and definite strong rays are produced (fig. 3). Where tetrarchy is established in the piliferous zone, the lateral roots may arise from any one of the four rays. More frequently, however, triarchy, with only three longitudinal rows of lateral roots (fig. 4) is evident up to the "collet" region. At this point triarchy, when present, invariably gives way to tetrarchy. In some cases a strong (fig. 9) and in others an abortive (fig. 5) fourth ray is developed. In either case, four very definite, alternating phloem groups are formed.

Instability in the number of root rays, according to COMPTON (2), is typical in many tribes of the Leguminosae, the number varying from two to four. It is noteworthy that in *M. sativa*, tetrarchy, although sometimes abortive with regard to the xylem ray, invariably becomes established as the final stage in primary root develop-

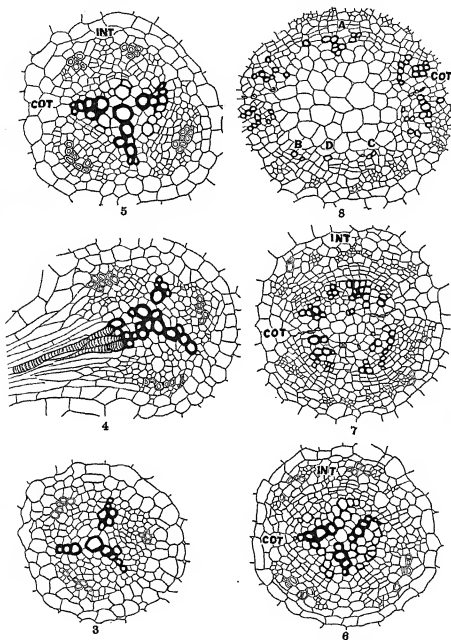
ment. When triarchy is present in the lower portion of the root, the three rays rotate their positions to allow for the development of a fourth. In the triarch stage the angles between the rays are practically equal and the phloem lies on alternate radii between them (fig. 3). Two of the rays, later identified as those in the cotyledonary plane and commonly called the polar xylems, gradually increase the angle between them until they come to lie end-to-end at an angle of 180° (fig. 5 *COT*). The third ray, now lying at right angles to them in the intercotyledonary plane (*INT*), becomes lateral. The fourth ray, when formed, also develops in the intercotyledonary plane, opposite the third ray. A fourth phloem group meanwhile appears, the original three having changed positions simultaneously with the xylem groups so as to retain their alternate positions (fig. 5). Tetrarchy persists through a considerable portion of the root. Seedlings just unfolded show tetrarchy for a distance of 0.75 mm. or more. Plants at the age of two weeks show this condition for a distance of 5 mm. Additional phloem groups, two flanking each of the original ones, arise in this region (fig. 6). They are the first evidences of the second or intermediate phase of CHAUVEAUD (1). When the original phloem groups are not distinctly differentiated, it appears at this stage as if there were eight groups, a pair lying at the outer edge of each xylem ray (fig. 9).

FORMATION OF COTYLEDONARY TRACES

The drawings represent selections from serial transverse sections of a 6-day-old plant. The sections progress upward from the point where the first evidences of tendencies to modify the tetrarch structure appear. The early dominance of secondary tissue in this region is shown by a few sections from a series of an 8-day-old plant (figs. 5-8). The cotyledonary axis (*COT*) and the intercotyledonary axis (*INT*) are shown throughout both series.

The gradual change in position of the vascular elements in the hypocotyl and the ultimate formation of the cotyledonary traces are shown by figures 9 to 18. The entire primary vascular system of the root is continuous with the vascular system of the cotyledons, each cotyledonary trace being made up of a polar and a part of each lateral xylem group, in the following manner. There is a gradual

spreading of the vascular elements toward the periphery of the stele. The outward movement is more pronounced along the cotyledonary axis of the hypocotyl. In transverse section, therefore, the



FIGS. 3-8.—Transverse sections selected from a series through root and hypocotyl of 8-day-old plant: *COT*, cotyledonary plane; *INT*, intercotyledonary plane. Fig. 3, triarch stage with three phloem groups. Fig. 4, formation of lateral root. Fig. 5, rotation of three xylem rays and formation of abortive fourth ray; appearance of fourth phloem group, cambium, and secondary tissue. Figs. 6-8, formation of cotyledonary traces; *A*, *B*, *C*, *D*, secondary vessels which are continuous with plumular traces to first leaves.

central cylinder assumes more and more the appearance of an ellipse with the polar rays on the long axis (figs. 13-15).

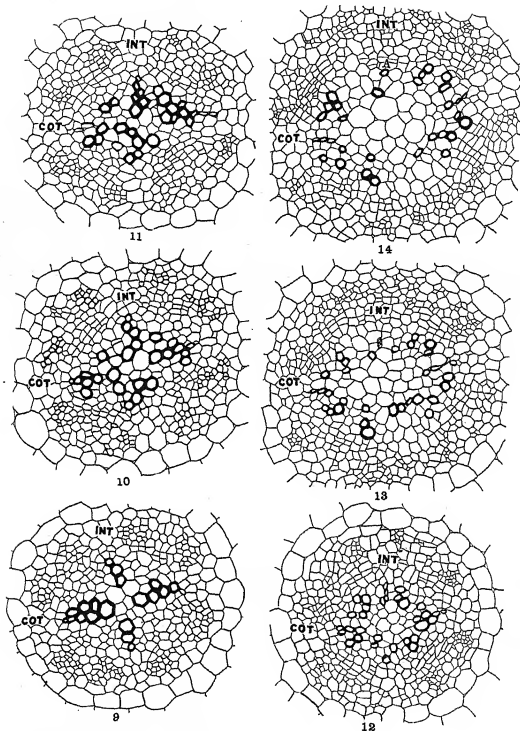
The metaxylem elements of the rays become somewhat separated from the protoxylem elements. Together with secondary vessels early arising from the cambium (figs. 6, 7), they form loose groups, one on either side of each protoxylem strand (figs. 10-12). External to each of the groups thus formed lies a phloem group, one of the eight previously mentioned. The polar protoxylem elements retain their identity and position in the cotyledonary plane throughout, although rapid elongation stretches and distorts them. This is especially evident as they near the cotyledonary node (figs. 13-15). The lateral protoxylem vessels retain their identity in the inter-cotyledonary plane (*INT*) for some distance, but eventually become disorganized and disappear (figs. 12, 13).

The groups of vascular tissue now constituting each of the inter-cotyledonary xylems continue to separate, curving toward the cotyledonary xylems so that, eventually, some vessels from each of the lateral xylems reinforce the xylem groups which lie on the right and on the left of the polar protoxylems (figs. 14, 15). The lateral phloem groups change position simultaneously with the xylem, and come to lie, together with the polar phloem, collateral to the two xylem groups on either side of the polar protoxylem (figs. 15, 16, 18).

The resulting structure constitutes the double bundle of THOMAS (5) or, more aptly, the triad bundle of COMPTON (3), being a protoxylem strand, flanked obliquely on either side by a collateral bundle. It is most distinctly seen at the base of the cotyledon where it definitely becomes the cotyledonary trace (figs. 16, 18). Thus the entire primary vascular system of the seedling, as well as some secondary tissues in older plants, is continuous with the vascular system of the cotyledons, each cotyledon receiving a triad (figs. 16, 18).

COTYLEDON

As the triad structure continues into the petiole of the cotyledon, the median protoxylem becomes disorganized and gradually disappears. A branch separates laterally from each bundle of the triad just before the disappearance of the median protoxylem, and the remaining portions of the double bundle fuse to form the collateral



FIGS. 9-14.—Selections from a series of transverse sections of a 6-day-old plant: *COT*, cotyledonary plane; *INT*, intercotyledonary plane; fig. 9, tetrarch stage, eight phloem groups; figs. 10-12, bifurcation of metaxylem; beginning of pith; fig. 13, disappearance of intercotyledonary protoxylem; elongation of cotyledonary axis; movement of intercotyledonary xylem toward cotyledonary poles; fig. 14, triad structure becoming obvious; appearance of *A*, which is continuous with plumular foliar trace. $\times 250$.

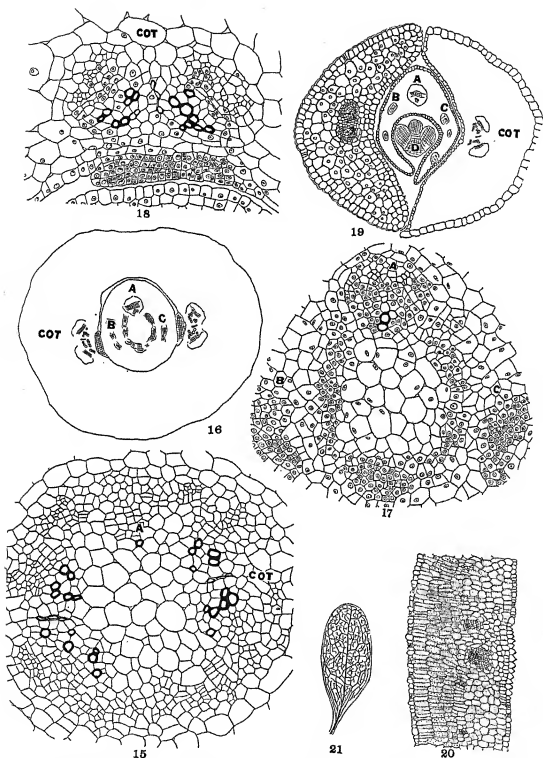
midrib strand of the cotyledon (fig. 20). Numerous other branch veinlets arise, forming an intricate system of venation (fig. 21). Thus, in the cotyledon lamina, the superposed phase of CHAUVEAUD (1) is the only phase to develop, recapitulation being accelerated and the earlier phases being suppressed.

PLUMULAR DEVELOPMENT

There is a decided pause between the development of the primitive vascular structure of the seedling and the superposed vascular structure of the plumule. The vessels arising from the procambium in the plumule are continuous with elements produced by the cambium in the hypocotyl and root. The first vessels in the hypocotyl which are continuous with vessels of the plumule are seen in the intercotyledonary plane at the point where the elements of the lateral xylem rays turn from their position to join the polar rays (figs. 14, 15 A). Thus these first vessels are superposed upon the seedling structure in the region of either the third or fourth xylem ray. In the latter case, when the fourth ray is largely an abortive one, they are especially evident in the intercotyledonary plane because of the absence of the elements of the fourth ray (figs. 7, 8 A). They are made up of collaterally placed xylem and phloem elements produced by the cambium in this region and, continuing upward, become the median trace to the first plumular leaf (figs. 16 A, 17 A, 19 A).

Three traces supply the first leaf, the median trace (A) already mentioned and two lateral traces (B, C). The two lateral traces arise on the side of the central cylinder opposite the median trace, one on either side of the intercotyledonary axis (figs. 8, 16, 17, 19 B and C). In a 6-day-old plant they appear as two groups of procambium (figs. 16, 17, 19). Before joining the median trace in the petiole of the first leaf, they give rise to numerous branches which supply the stipules (fig. 19). The median trace to the second leaf lies opposite the median trace of the first leaf on the other side of the vascular cylinder (figs. 8, 19 D).

The vascular cylinder of the plumule of the seedling consists of only meristematic tissue or at the most of a few vessels and procambial strands. The foliar traces become separated from the vascular cylinder less than 0.5 mm. above the cotyledonary node (fig. 19).



FIGS. 15-21.—Figs. 15-19, transverse sections of a 6-day-old plant continued from fig. 14: fig. 15, cotyledonary node before separation of cotyledons from main axis of plant; fig. 16, diagram of cotyledonary node: *COT*, cotyledon; *A*, *B*, *C*, traces to first leaf, bud in axil of cotyledon; figs. 17, 18, detail of fig. 16: fig. 17, plumule; fig. 18, triad bundle of cotyledon; fig. 19, cotyledons separated from plumular bud which lies between them; fig. 20, cross-section of cotyledon lamina; fig. 21, cotyledon. Figs. 15, 17, 18, $\times 250$; figs. 16, 19, 20, $\times 50$; fig. 21, $\times 3$.

It is evident that the plumule of the seedling is merely a bud between the two cotyledons.

In older plants evidences of the priority of development of traces to the first and second leaves are lost because of the strong development of secondary vascular tissues. These traces become merely bundles separating from the more or less continuous vascular cylinder much in the manner of subsequent leaf traces. All the primary vascular vessels become imbedded in secondary tissue.

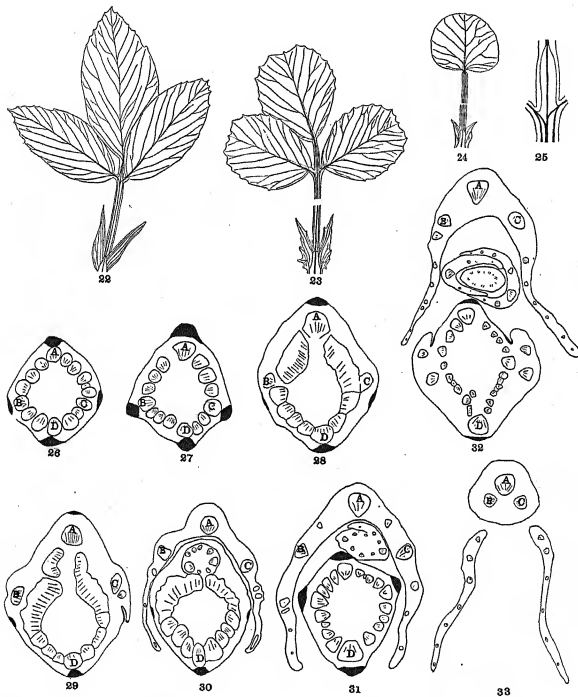
MATURE SHOOT

The first leaf is unifoliolate (fig. 24) and all subsequent leaves are trifoliolate (figs. 22, 23). The stipules at the bases of the leaves are prominent and leaflike. The internode between the first and second leaf is very short in the bud, but elongates greatly in the maturing plant. A bud lies in the axil of each cotyledon (figs. 16, 18) and in the axil of each leaf. These buds, together with the plumule, initiate the stems which arise from the crown of the plant.

Three vascular bundles constitute the foliar traces in both unifoliolate and trifoliolate leaves. The two lateral traces furnish veinlets for the two stipules (figs. 22-24). The median trace enters the petiole directly without branching. The three traces anastomose at the point where the stipules become distinct from the petiole but separate again and are seen in the petiole as three distinct bundles (figs. 22-24).

The unifoliolate leaf is similar to the trifoliolate leaves in vascular structure of stipule and petiole up to the point where the bundles enter the leaflets. At this point, in the unifoliolate leaf, the three bundles from the petiole fuse and enter the one leaflet as a single bundle (fig. 24). In the case of the trifoliolate leaf, the terminal leaflet receives the median bundle and each of the two lower leaflets receive a lateral bundle. However, just as the two lateral bundles turn to enter the two lower leaflets, each is reinforced by a strand from the median bundle and at the same time each sends a strand to join the median bundle. The actual junction of the latter does not occur, however, until the median bundle enters the terminal leaflet (fig. 25). The leaflets are pinnately net-veined (figs. 22-24). Details of venation, showing branching to vein islets, are illustrated by WILSON (7).

The change in the shape of the leaf blade from the cordate outline of the first formed leaflets to the obovate outline of the mature



FIGS. 22-33.—Fig. 22, mature leaf with stipules attached; fig. 23, young leaf; fig. 24, unifoliate leaf; fig. 25, vascular system of petiole as it passes into the three leaflets; figs. 26-33, successive diagrams showing departure of leaf and bud traces: A and D, median foliar traces; B and C, lateral foliar traces.

leaflets is a feature which *M. saliva* shares in common with many of the Trifolieae, according to COMPTON (2) (figs. 22, 23).

The origin of the vascular strands which form the traces to the leaves and branches is represented by successive transverse sections of the stem (figs. 26-33) and by a schematic diagram of four nodes of the stem (fig. 34). In figure 34 the internodes are shortened and the width of the trace bundles much reduced.

The phyllotaxy being alternate, the three leaf traces alternate from node to node. The bundles constituting the median leaf traces (*A* and *D*) are broad (figs. 26-32). They originate in two series, being formed on opposite sides of the stem by the union of two strands, one from each of a pair of neighboring stem bundles (fig. 34). These stem bundles retain their identity from node to node, giving rise to successive trace bundles at every other node. Thus the bundles which form the median leaf traces on either side of the stem become identities in the vascular cylinder two nodes below their point of departure (fig. 34). Departure is somewhat abrupt, the stem becoming considerably enlarged on the trace side.

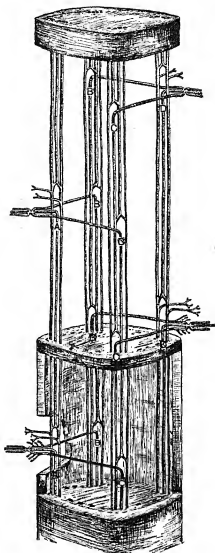
Only one stem bundle gives rise to each of the two series of lateral leaf traces. These stem bundles lie on the vascular cylinder at right angles to the axis on which the median bundles arise, or, in other words, one-fourth of the way around the central cylinder from either median bundle. A strand separates from each of the stem bundles at each node and becomes a lateral leaf trace at the node above (fig. 34 *B* and *C*). These strands (*B* and *C*) therefore become definite bundles in the vascular ring at only one node below their point of departure. They alternate from side to side at successive nodes (fig. 34).

At each node, traces to the branch bud which lies in the axil of the leaf arise just above the departed median leaf trace from the identical pair of vascular stem bundles which form the median leaf traces (fig. 34).

Figure 26 represents the stem cross-section in the region of the internode just below a node. Bundle *A* is the median foliar trace bundle. Bundles *B* and *C* are the lateral foliar trace bundles. In figure 27, the traces have separated slightly from the stem cylinder. As separation continues (figs. 28-32), the portion of the stem in

which these bundles lie increases in size and finally becomes distinct from the stem. The three foliar gaps caused by the departure of the three traces are closed above. Figure 33 represents a transverse section through the petiole and stipules at a higher level, where *A*, *B*, and *C* are petiolar bundles. The vascular strands shown in the stipules are merely smaller branches arisen from the lateral trace bundles (*B* and *C*). Just above the point where the median leaf traces separate from the stem axis, the branch traces also leave the vascular ring (figs. 28, 29). The gap formed by their departure is promptly closed. They form the miniature vascular ring of the bud (fig. 30). The young stem initiates petiolar and stipular formation in the exact manner of the parent stem (figs. 31, 32).

At the time the bundles *A*, *B*, and *C* have become distinct from the main axis, the alternating median trace bundle (*D*) becomes evident opposite bundle *A*. Two lateral bundles, corresponding to *B* and *C*, also separate from the main axial cylinder. The process of stipular, petiolar, and branch formation proceeds as indicated but on the opposite side of the stem (figs. 30-32). WILSON (7) states, "the corners of the somewhat four-angled stem show a multiplication of the cell layers under the epidermis seemingly serving for greater strength." These



34

FIG. 34.*—Schematic diagram of vascular system of stem showing four nodes: internodes shortened and width of vascular bundles much reduced; *A* and *D* are median leaf trace bundles, *C* and *B* are lateral trace bundles; the three traces anastomose before entering the petiole; the lateral traces give rise to branches which supply the stipules; branch traces depart above leaf traces.

* Drawn for this study by JOHN MACK WINTER.

cells are collenchymatous. They occur at the corners opposite each trace bundle (fig. 26).

More or less anastomosing occurs at the node on the side of the departing traces. Between nodes there are no anastomoses. The pattern of the vascular system is seen, therefore, to be comparatively simple as it passes through the stem.

Discussion

In a study of *Medicago sativa* it is found that the primary vascular system of the root, hypocotyl, and cotyledons forms a complete circulatory system of its own, independent of subsequent plumular development. This situation is in agreement with COMPTON'S (2) generalization for the vast majority of epigeal seedlings. The primary system recapitulates the phases of evolutionary development of vascular tissues as described by CHAUVEAUD (1) in his phanerogamic cycle, passing from root to cotyledon through the exarch, alternate arrangement to the intermediate phase, and finally to the endarch, collateral, or superposed condition.

The appearance of the vascular elements in the hypocotyl region can best be interpreted by the recapitulation theory of CHAUVEAUD. The radial arrangement of vascular elements, or the alternate phase, persists throughout the region, being especially evident in the cotyledonary plane. Metaxylem elements, added more or less tangentially on either side of the protoxylem, illustrate the intermediate phase of development. Almost simultaneously with this metaxylem formation, collateral elements arise, further augmenting the vascular system and illustrating the superposed phase. In older plants the primary tissues, constituting the first two phases, become disorganized, and collateral, secondary tissues become the dominant ones. The capacity for the production of the more primitive phases seems to be transient in this region.

Interpreted by CHAUVEAUD'S theory, the singular structure constituting the cotyledonary trace ceases to be an anomalism. The median protoxylem strand is a recapitulation of the primitive alternate phase. The flanking bundles are the intermediate and superposed phases. The development of the two earlier phases is suppressed only in the cotyledon lamina.

When the plumular vascular elements develop slightly later they are collateral and endarch, and are directly continuous with secondary elements formed by the cambium in the region of the hypocotyl. The inception of the plumular elements is thus marked by the emergence of only the more advanced type of vascular tissue, the more primitive phases being apparently arrested. As development continues, secondary vascular tissues rapidly assume prominence and a direct connection is established by them from stem, leaves, and branches, downward into hypocotyl and root. A continuous ectophloic siphonostele becomes the dominant conducting system through root and shoot. Even at the growing tip of the root where the primitive radial arrangement continues to form, secondary vascular elements laterally supplement the primary elements.

Thus the problem of continuity presented by the "transition" region is solved without great difficulty, for the primary vascular system of the root does not communicate directly with the more advanced vascular arrangement of the shoot. The seedling, with its temporary, leaflike cotyledons may be a recapitulation of the primitive ancestor of *M. sativa*. Certainly the superposed secondary system belongs to a later evolutionary period.

Summary

1. The primary vascular root system of *Medicago sativa* L. is triarch or, more rarely, tetrarch. Lateral roots arise directly opposite the xylem strands.
2. Triarchy in the root, when present in the lower portion, changes to tetrarchy in the hypocotyl region, although the fourth ray may be an abortive one.
3. Each cotyledonary trace is made up of a polar xylem strand and a part of each lateral xylem strand. Cotyledonary traces, as they pass into the cotyledons, are composed of triad bundles, the median portion being a polar protoxylem strand.
4. The median protoxylem strand of the cotyledonary trace disappears as the triad bundle approaches the cotyledon lamina. The two remaining flanking bundles fuse to form the collateral midrib bundle of the cotyledon blade.
5. The primary system of the root, hypocotyl, and cotyledons

forms a complete circulatory system of its own, independent of subsequent plumular development. It recapitulates the phases of evolutionary development as described by CHAUVEAUD in his phanerogamic cycle.

6. When the plumular elements develop, slightly later, they are collateral and endarch and are directly continuous with secondary elements formed by a cambium in the region of the hypocotyl.

7. The first plumular vessels to form are those which become traces to the first and second leaves. These leaves arise at alternate nodes above the cotyledonary node. Later, the formation of a complete cylinder of bundles in the stem obscures the priority of the emergence of these first trace bundles.

8. Secondary tissues early form a continuous conducting system through root and shoot. The primary tissues become imbedded in secondary tissues except at the tip of the root where they are laterally supplemented by secondary tissue.

9. The pattern of the vascular system in the stem is comparatively simple, being the result of three foliar traces alternating from node to node and of two branch traces supplying the branch which forms in the axil of each leaf.

10. Six stem bundles are principally involved in bud and leaf trace formation. These bundles partially lose their identity at the nodes because of anastomoses of all the bundles on the side of the stem where the leaf is forming.

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MULTIPLE MALE CELLS IN CUPRESSUS ARIZONICA

CLIFTON C. DOAK

(WITH SEVENTEEN FIGURES)

Introduction

In spite of extensive work on the morphology of members of the Cupressineae, many uncertainties still remain. The following work on *Cupressus arizonica* was therefore begun in 1929. The present paper deals with a description of the male gametophyte.

MATERIAL AND METHODS.—The material was gathered in the spring of 1929 from a single tree, which is one of a small group growing in the grounds of Texas Agricultural and Mechanical College at College Station, Texas. The tree was approximately 25 years old and seemed to be in perfectly normal condition.

The nucellus, together with the inclosed female gametophyte, was carefully dissected from the hard seed coat while the material was submerged in 0.3 gm. molecular cane sugar solution, this having been successfully used by BUCHHOLZ (1) for the dissection of embryos of coniferous plants. Formalin acetic alcohol was used as a killing solution. The material was imbedded in paraffin, cut at 10μ , and stained in safranin and fast green, which gave good detail of nuclear and protoplasmic structures. Attempts made to section entire ovules were unsuccessful, due to the hard seed coats.

Investigation

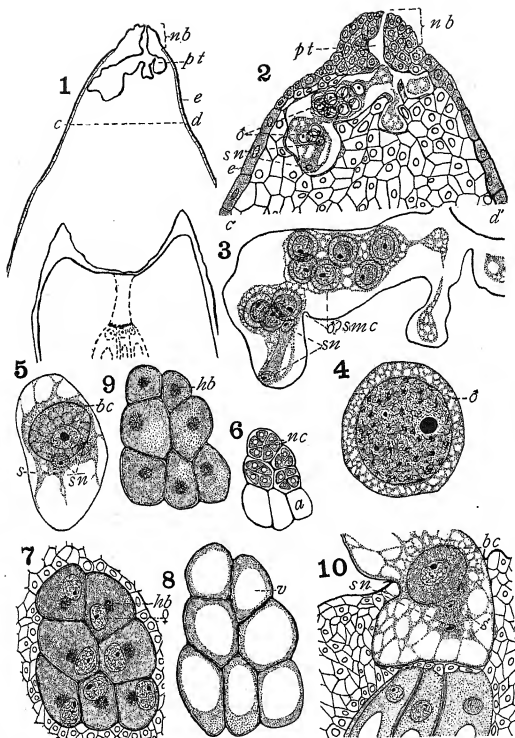
The nucellus is a conical structure, and is free from the integument to a point far below the top of the female gametophyte. The cells in this "nucellar beak" are smaller and have a greater protoplasmic content than have the cells in other regions (figs. 1, 2). The epidermal cover is discontinuous over the tip of the nucellus, and here the surface is somewhat irregular. The lower portion of the nucellus is covered with an epidermal layer which stains deeply with safranin (fig. 2 e). This layer is resistant to the action of the pollen tubes, and restricts their growth beyond the limits of the nucellus.

POLLEN GRAINS AND POLLEN TUBE

The wingless pollen grains of members of the Cupressineae are shed in the microspore stage. There are never any prothallial cells. According to COULTER and CHAMBERLAIN (5), this condition is known in at least seven of the ten genera in this group, including *Cupressus*.

The first division of the microspore nucleus gives rise to the generative and tube nuclei. As shown by COKER (4), this takes place after the pollen is shed on the nucellus and before the tube is put out. Later a tube is formed and both nuclei enter it. At first the tube is small, but in the nucellus it usually widens rapidly and becomes evaginated and branched at various points, following a devious course (figs. 1, 2 *pt*). In a few cases the tube was found to be rather smooth and to follow an almost direct course; but even in such cases it widens greatly in the course of advancement, and upon entering the archegonial chamber soon broadens to fill this space completely (fig. 10). Within this tortuous and somewhat conical tube, the generative cell divides to form the stalk and body cells. The body cell increases enormously in size (figs. 5, 10, 16), and finally gives rise to the male cells. In most members of this genus these are limited to two which are equal in size and both functional. In the present material no very early stages were found. The youngest (June 13) shows the stalk and body cells already formed.

Many different developmental stages may be found at any given time, indicating considerable variation in the rate of growth and in the behavior of the different pollen tubes. Variation is also found in the time at which the division of the body cell takes place, as well as in the number of divisions which finally ensue. In some cases the undivided body cell was seen lying in the tip of a greatly swollen pollen tube which was already in contact with the necks of mature archegonia (fig. 10). In other instances, tubes in a similar position contained two equal, large, hemispherical cells which in every way resembled the two male cells so often described and figured for members of this genus. Another tube which had reached the archegonial chamber contained twelve curiously shaped "sperm cells" (figs. 14, 15 *b*). Often the division to form many male cells takes place long before the archegonial chamber has been reached. In one



FIGS. 1-10.—Fig. 1, longitudinal section of nucellus with ramified pollen tube; position of archegonia indicated in dotted lines below; $\times 162$. Fig. 2, upper part of same, showing content of pollen tube built from serial sections; cytoplasmic content of male cells omitted; $\times 150$. Fig. 3, more highly magnified view of best section from which fig. 2 was reconstructed; $\times 300$. Fig. 4, more highly magnified view of one of male cells shown in figs. 2 and 3; $\times 1650$. Fig. 5, cross-section of swollen end of pollen tube which filled archegonial chamber and was in contact with neck cells. Figs. 6-9, sections taken from different levels of same specimen; figs. 7 and 9 partly reconstructed from serial sections. Fig. 6, archegonial neck cells in face view. Fig. 7, cross-section of upper end of archegonial complex showing egg nuclei, each accompanied by a Hofmeister body or "asteroid." Fig. 8, median cross-section of same complex showing characteristic large vacuoles. Fig. 9, basal section of same; Hofmeister bodies or "asteroids" present in each archegonium. Fig. 10, longitudinal section of upper end of female gametophyte with pollen tube broadened to fill archegonial chamber; figs. 5-10, $\times 162$.

case (figs. 1-3) a tube which had traversed less than one-third the distance to the archegonial chamber was seen to contain fourteen cells (possibly sperm mother cells).

MULTIPLE FERTILIZATIONS

Several types of evidence indicate that a great number of male cells from a single tube often participate in many separate fertilizations:

1. In spite of the fact that usually only one or two tubes enter an archegonial chamber, fertilization of the eggs in numerous archegonia of a single complex may take place almost simultaneously.

2. Often more than one male cell could be seen in a single archegonium (fig. 13), indicating that had the tube content been distributed differently more eggs could have been fertilized.

3. The contrast in size of the undivided body cell and its nucleus, in comparison with male cells participating in fertilization, would suggest that not half but a smaller fraction of this material goes into the construction of the functioning male cell. This may be seen by comparing the body cells shown (figs. 5, 10, 16) with the functional male cells (figs. 13, 15 b, 17).

4. There is some indication that the presence of one tube in position above the archegonial chamber tends to inhibit the entry of others, thus limiting the fertilizations in a single archegonial complex to the male cells from a single tube. A case was found in which one tube was in position above the archegonia, and another had traversed the tissues of the female gametophyte much as it had traversed the nucellus (fig. 15 a). A tube in this position would be at a great disadvantage, for there is evidence that the tube has not the enzymes necessary to penetrate the walls of the archegonia. For example, during normal entry, at which time the tube readily digests the neck cells, it leaves the upper walls of the archegonia intact. Even if one of these lateral tubes should effect an entry, it would gain access to only one or two of the archegonia. These lateral tubes were first seen by HOFMEISTER (7). He reported a case observed in *Taxus*, in which a number of such tubes twined about the archegonia in such a way as to shut off completely the source of nourishment to the archegonia.

5. Another indication of plurality of fertilizations by a single tube comes from the fact that, from data gathered for a forthcoming paper on the embryology of this species, I find that the number of zygotes per complex is in excess of what would be expected on a basis of two fertilizations per pollen tube.

6. As already pointed out, when there is more than one pollen tube growing within a single nucellus, their cellular contents are usually in widely different stages of development. Thus any eggs which remain unfertilized by the first tube would probably have to retain their ability to function for a considerable time while waiting for the maturity of the other tubes. There is abundant evidence that this is not the case, for after proembryo development has started within a part of the archegonia of an archegonial complex, the adjacent archegonia which do not contain proembryos rapidly disintegrate.

7. If the separate archegonia of a complex are served by tubes which vary considerably in their time of maturity, one would expect the resulting zygotes and embryos to vary proportionately as much as do the pollen tubes which initiate them. On the contrary, these structures are always in approximately the same developmental stages.

It seems safe to conclude, therefore, that the many male cells of a single pollen tube of *Cupressus arizonica* fertilize a number of eggs in the archegonia within an archegonial complex, and that in most cases the work of fertilization within a single complex is limited to the male cells from a single pollen tube.

MALE CELLS

It has been pointed out that two hemispherical cells similar to those commonly found in other Cupressineae were seen in tubes already in contact with the necks of the archegonia, suggesting that the two functional male cells so commonly described for other species are found here also. However, these may represent cases of delayed division of the body cell. That such delay does occur is attested by the presence of undivided body cells even in pollen tubes which have already reached the archegonial chamber (figs. 5, 10). It is therefore impossible to say with certainty whether the two

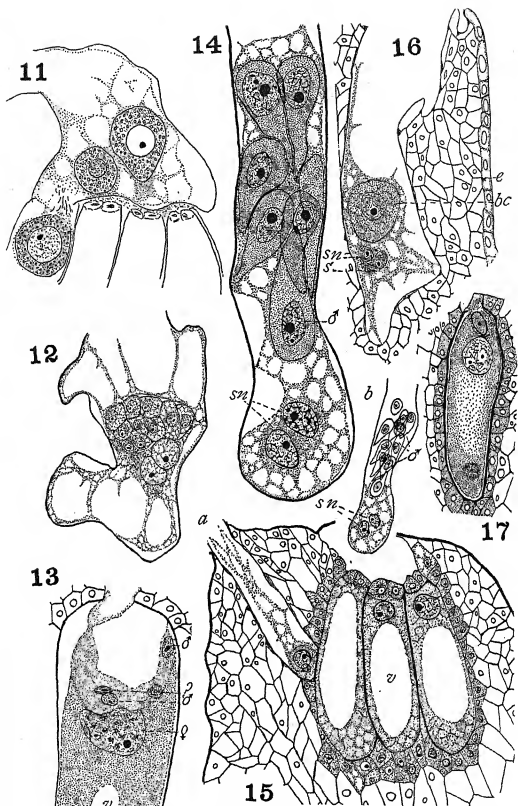
cells referred to participate in fertilization or first undergo further divisions.

In tubes which show the multicellular condition, the history of the male cells seems to indicate that the very large body cell (60–80 μ) divides rapidly and repeatedly to form a complex of from four to about fourteen male cells. At first the male cells are irregular in shape and lie together in a compact mass. It was in this condition that JUEL (9) delineated them in his figures, one of which is reproduced here as figure 12. JUEL shows this figure in series with a younger tube with undivided body cell and older tubes showing male cells which are slightly separated from each other and which have grown to approximately twice the size of the ones shown here. This increase in size makes the mature male cells as seen by JUEL in *Cupressus goveniana* approximately equal in size to those found in *C. arizonica*. Later the cells increase in size, round up, and separate from each other (figs. 2, 3). One tube was found in which there was a complex of male cells in which nearly all the cells were of a peculiar, elongated, pear-shaped form. Each had its own cytoplasm and was definitely bounded by a membrane (figs. 14, 15 b). This peculiar shape was not seen in earlier stages (figs. 2, 3) nor in male cells which had already entered archegonia (figs. 13, 17). Since this condition occurs here but once and not at all in the material of previous investigators, it is probable that this differentiation of the male cells into pear-shaped "sperm cells" is both evanescent and rare.

In addition to the tube and stalk nuclei and the male cells, the pollen tube contains a considerable amount of cytoplasm which is usually densest in the vicinity of the sterile nuclei (figs. 2, 3, 5, 10, 16). Frequently a few starch grains may be found in this cytoplasm (figs. 10, 16 s'). When present, these grains are usually closely associated with the sterile nuclei. The starch is never abundant, as reported for many of the Cupressineae.

The number of archegonia in a complex ranges from three to ten, with six to eight occurring most frequently. A single pollen tube may come in contact with the necks of all the archegonia in a complex (fig. 10).

In entering the archegonia, the tube destroys or displaces the neck cells (figs. 13, 17). Just before entry, the mature egg nucleus



FIGS. 11-17.—Fig. 11 (from NICHOLS, fig. 94), “tip of a pollen tube which contained one small and two large male cells. One of the latter has already entered an archegonium.” $\times 300$. Fig. 12, from JUEL, fig. 2 (see text). Fig. 13, single archegonium in longitudinal section, showing presence of more than one nucleus from pollen tube; $\times 300$. Fig. 14, best section from series from which fig. 15 *b* was reconstructed shown under higher magnification; seven of the twelve pear-shaped male cells (“sperm cells”) shown; $\times 365$. Fig. 15, longitudinal section of upper part of female gametophyte which has pollen tube (*a*) piercing the tissues; fig. 14 and the tube shown at *b* are from same specimen and represent a tube which had pierced the megaspore membrane as shown at *b*; $\times 127$. Fig. 15 *a*, pollen tube which has pierced the tissues of the female gametophyte. Fig. 15 *b*, mature pollen tube with content of twelve male cells reconstructed from serial section; cytoplasmic content of male cells omitted; $\times 137$. Fig. 16, longitudinal section of pollen tube with large body cell still undivided; $\times 325$. Fig. 17, archegonium just prior to fertilization: only single male cell entered.

is at the upper end of the archegonium and is surrounded by dense cytoplasm, which except for possible "HOFMEISTER bodies" is rather uniform in consistency. At this stage the large vacuole (fig. 15 v) is usually still present but is later filled by the addition of new cytoplasm to the archegonium. It has usually disappeared completely at the time of fertilization (fig. 17).

Discussion

HOFMEISTER, writing several years before the appearance of STRASBURGER'S work on fertilization in gymnosperms (20), did not clearly understand the rôle of the nucleus in fertilization. However, he evidently saw pollen tubes with both the usual two male cells and also others with the multicellular condition. He states, "In other cases the pollen tube contains four middle-sized or eight smaller, roundish cells without any firm membrane," and cites Pl. LXIV, fig. 3. This figure portrays a pollen tube in which seven nuclei are clearly shown. HOFMEISTER was near the truth as regards the fertilization of many eggs by the many male cells of a single pollen tube. He says, "In *Taxus* the impregnation of several corpuscula (archegonia) by the very widely expanded end of a single pollen tube is of very frequent occurrence and in *Juniperus* and *Thuja* it is the rule."

STRASBURGER speaks of the repeated division of one of the nuclei in the end of the pollen tubes of the Cupressineae and the distribution of the resulting cells over the necks of the archegonia. He states that in the Cupressineae a pollen tube fertilizes numerous eggs, and that a single nucleus is always seen above each archegonium before fertilization.

Preceded by these investigators, JUEL (9), working with *Cupressus goveniana*, was first to observe and completely interpret the plurality of male cells ("spermzellen") in the pollen tube of a conifer. In spite of the fact that other workers, notably NORÉN (13), NICHOLS (12), and SAXTON (18), have found evidences of irregularities in the number, manner, and time of male cell formation, it can be said that after nearly 30 years of more or less intensive work on the Cupressineae and closely related plants, JUEL'S work has stood, until now, unconfirmed. In speaking of the work of JUEL, COULTER and CHAM-

BERLAIN say that, "At present no conclusions are safe in regard to such rare occurrences."

Even as early as 1907 the view that JUEL's findings represented interesting abnormalities had already begun to be current, and this view has frequently been expressed since that time. LAWSON (11), after having described the two male cells as he found them in *Libocedrus decurrens*, points out that almost exactly the same conditions obtain in regard to the two species of *Cupressus* examined by him. He then adds that, "From LAND'S (10) account of *Thuja*, COKER'S (3) account of *Taxodium*, and my own observations of *Cryptomeria*, *Libocedrus*, *Thuja*, *Cupressus*, and *Chamaecyparis* there seems to be a striking uniformity throughout the Cupressineae in regard to the history of the male gametophyte." If LAWSON were writing today, he could strengthen his argument in favor of the normality of two equal male cells by adding to the above list, OTTLEY'S (14) account of *Juniperus communis* and *J. virginiana*, NICHOL'S (12) account of *J. communis*, and possibly others. For the Cupressoideae and Callitroideae he could add SAXTON'S work with *Widdringtonia cupressoides* (16), *Callitris* (17), *Actinostrobus pyramidalis* (18), and *Tetralix articulata* (19). In this body of work, however, at least three writers, NORÉN, NICHOLS, and SAXTON, reported having seen more than two male cells in a single pollen tube. Although little was made of these observations, the evidence is sufficient to show that this condition did occasionally occur in their materials, and that the male cell number is not rigidly fixed at two in all members of the Cupressineae.

LAWSON seems either not to have been familiar with the works of HOFMEISTER (not cited) and STRASBURGER, or else unwilling to accept their obvious implications, for in defending the position that the organization of two male cells of equal size is a constant character of the Cupressineae, he says that, "The only exception which has so far been recorded is that of *Cupressus goveniana*, in which JUEL described the body cell as giving rise to a complex of cells."

The present study confirms in nearly every detail the work of JUEL on *Cupressus goveniana*, and at the same time adds some significant stages not seen by him. The most prominent of these new features is the partial differentiation of certain of the male cells into "sperm cells."

In seeking to account for the peculiar shapes assumed by these cells (fig. 14), their similarity in outline to certain zoospores or to the sperm cells of cycads and *Ginkgo* recalled HOFMEISTER's reference to the possibility of sperm cells in the pollen tubes of the Coniferae.

A search of the literature was made to see whether male cells with similar shapes had been seen before. In a few cases there is a suggestion of such a thing; but in most of these the elongated shapes are such that they could be accounted for by the recent mutual pressure of the newly formed hemispherical male cells which had simply not yet fully rounded out. SAXTON (19) reports that the male cells of *Tetraclinis articulata* retain their hemispherical shapes even after long separation from each other. NICHOLS (12, Pl. XVI. fig. 94, reproduced here as fig. 11) shows a pollen tube of *Juniperus communis* with three male cells, which according to him represents an abnormal condition for this species. Two of these cells are slightly elongated. It would appear that NICHOLS saw a suggestive shape in the male cells of a pollen tube, which also had supernumerary male cells. Perhaps it is a mere coincidence that these were in a tube which had more than the usual number of male cells, and which had reached the same stage in its ontogeny as the tube which contained the pear-shaped cells in my material. In speaking of the matter of cilia, NICHOLS states:

The demonstration of blepharoplasts in the body cells of the Ginkgoales and Cycadales suggests the possibility that some traces of cilia-forming organs might exist among the Coniferales and Gnetales. A careful study, however, of a large number of body cells in *J. communis depressa*, in all stages of development, has failed to reveal any structures which appear definitely homologous with blepharoplasts.

JUEL's figures were all taken from material which was either too young or too closely compacted to show these elongated shapes.

HOFMEISTER predicted the finding of swimming sperms in the pollen tubes of the gymnosperms. HIRASÉ (6) partly fulfilled the prophecy by announcing their discovery in *Ginkgo*. IKENO (8) added *Cycas revoluta*, and WEBBER (21) added *Zamia integrifolia*. HOFMEISTER at the time of his prediction, however, was concerned neither with *Ginkgo* nor with a cycad, but with various higher conifers, especially the members of the Cupressineae. Although no claim is made that cilia have been found, the evidence is such that new

hope is raised for the ultimate fulfillment of HOFMEISTER's prediction.

The pollen tube of the angiosperms, because of the phenomenon of double fertilization, is not exactly comparable with that of the gymnosperms. The fact remains, however, that the former contains two functional male nuclei while in many gymnosperms the number is reduced to one. A second nucleus is present in various stages of development but is non-functional. Among many of the Cupressineae, the functional number seems to be more or less fixed at two. In many cases the stabilization is not complete and members of the Cupressineae develop tubes with more than two male cells. In certain still less stable forms, notably *C. goveniana* and *C. arizonica*, two male cells may be produced occasionally, but more regularly they produce a complex of several which in some cases become differentiated into "sperm cells."

The question arises as to whether these characters in *Cupressus* are palingenetic or coenogenetic. JUEL favors the palingenetic view. He holds that, since the gymnosperms in general can accomplish multiple fertilizations simply by growing a plurality of pollen tubes, and since only one embryo can come to maturity, multiple fertilizations through plurality of male cells would therefore have no survival value, and could not have arisen out of a form which produced but two male cells. JUEL overlooked the possible advantages of internal embryonic selection, or of secretory effects of multiple suspensors which BUCHHOLZ (1) later pointed out might account for the prevalence of polyembryony among conifers. JUEL concludes that we must assume for the Cupressineae a phylogenetic line which has preserved the multicellular condition. He holds that the Cupressineae and the other conifers split off very early from a common ancestor and have developed in parallel lines.

COULTER and CHAMBERLAIN take the opposite view. They state:

While phylogenetic continuity of multiple sperms is safely claimed for *Microcycas* (CALDWELL 2), no such claim could be maintained for Cupressineae.

In speaking of a single versus a plurality of sperm mother cells they say,

It is interesting to note that the very few instances of a greater number of sperm mother cells occur in a group characterized by its retention of ancient features (Cycadales), and in another group characterized by its very modern features (Cupressineae).

Phylogenetically an organism, or group of related organisms, is made up of an assemblage of many evolving structures which, despite the fact that the parts may not evolve at equal rates, for questions of phylogenetic position must be considered in their entirety. When it is so considered, the group making up the Cupressineae is unquestionably advanced, not only in regard to the sporophyte and female gametophyte, but in regard to the vegetative tissue of the male gametophyte itself. The writer is therefore inclined to the position that the cupressinean assemblage is advanced, and that the male cell complex found in certain members of the Cupressineae is a reversion which was made possible in a limited number of forms by the accidental sequence of evolutionary development between two structures which were somewhat interdependent, that is, the archegonial complex and the male cell complex. It would seem that in the female gametophyte the condition of scattered archegonia is primitive and the archegonial complex advanced, and that for the male gametophyte a plurality of sperm cells is primitive and the reduced number advanced. In ancient forms which existed before the advent of the pollen tube as a sperm-conveying mechanism the sperms moved from the spermagonium to the archegonium through their own motility. Under such conditions, in spite of the scattered archegonia, the sperms from a single spermagonium could readily fertilize the eggs in widely separated archegonia. Without claiming the *Cordaianthus* as a direct ancestor for the Cupressineae, attention is called to the pollen grain of *Cordaianthus grand'euryi* with its many hundred cells (as figured by RENAULT 15) as probably representing this level in evolutionary development. The establishment of the pollen tube as an organ of transfer of male cells to the archegonia rendered obsolete all male cells in excess of one, and eliminated the necessity for cilia. Evolutionary reduction set in, and certain forms, such as *Taxus*, *Pinus*, *Abies*, etc., in which the archegonia have remained permanently separated, show the evolutionary reduction in the male gametes as it approaches its final culmination in a single functional male nucleus. In certain forms, however, represented by *Juniperus*, *Biota*, and *Thuja*, in which the female gametophyte produces an archegonial complex, the male nuclei of the pollen tube could fertilize a plurality of eggs. This condition favored those pollen tubes which contained the largest number of male cells, and brought about a

return to, and a re-establishment of, the primitive condition of multiple male cells. In most Cupressineae the pollen tube seems to have been stabilized at two male cells before the formation of an archegonial complex. In *C. goveniana* and *C. arizonica* the pollen tube, by virtue of its greater variability, reverted to the habit of forming a male cell complex. Since the pollen tube is retained as a gamete-conveying organ, it is not to be expected that the reversion will extend to the fixation of ciliated sperms as a regular habit; but that these, if seen at all, will represent the extreme of the atavistic tendency which for lack of survival value has not, and possibly will not, become permanently re-established.

Summary

1. The ontogeny of the male gametophyte of *Cupressus arizonica*, with the exception of the early stages, is reported.

2. There is great irregularity in the behavior of the pollen tube and in the time of its development.

3. Some evidence is cited to support the view that two equal, functional male cells may occasionally occur.

4. In most cases a pollen tube develops a complex of several male cells, and occasionally these are differentiated into "sperm cells" with distinctive shapes, suggesting possible motility.

5. Although blepharoplasts were not seen and the evidences of cilia were not conclusive, the possibility is suggested that ciliated sperms may be found among the higher conifers. The complete return to ciliated sperms as a regular habit is not regarded as likely, but may occur as representing the extreme of an atavistic tendency.

6. Evidence is given to support the view that the many male cells of a single pollen tube serve to fertilize a number of different eggs in a number of separate archegonia of the complex.

7. The male-cell complex is considered to be a reversion. The trend toward reduction was reversed when the development of an archegonial complex made possible multiple fertilizations from a single pollen tube.

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ECOLOGICAL ASPECTS OF TRANSPIRATION

II. PIKE'S PEAK AND SANTA BARBARA REGIONS: EDAPHIC AND CLIMATIC ASPECTS

CHARLES J. WHITFIELD

(WITH SIX FIGURES)

Introduction

The first part of this research (5) dealt with the functional responses of plants and the measurement of physical factors in the main climatic associations of the Pike's Peak region, Colorado (6). In addition the vegetation was briefly described, and details of the phytometer and instrumental methods employed in these investigations discussed. The present paper deals with the edaphic aspects of the Pike's Peak region, and the edaphic and climatic relations of Santa Barbara County, California.

In Santa Barbara County, investigations were made in the coastal sagebrush and the coastal chaparral associations, and in the sand dunes along the coast (1, 7). The conspicuous dominants of the coastal sagebrush are *Artemisia californica*, *Salvia mellifera*, *S. leucophylla*, *S. apiana*, and *Eriogonum fasciculatum*. The principal chaparral dominants are *Adenostoma fasciculatum*, *Quercus dumosa*, *Cercocarpus parvifolius*, and species of *Arctostaphylos* and *Ceanothus*. The most abundant dune species are *Lupinus chamissonis*, *Abronia maritima*, *Franseria bipinnatifida*, *Solanum douglasii*, *Heterotheca grandiflora*, and *Convolvulus soldanella*.

The climate of Santa Barbara County is equable throughout the year, excessive heat and cold being very rare. The average seasonal rainfall from 1925 to 1930 was 15.68 inches, and was confined to the winter and spring months.

Results

PIKE'S PEAK REGION

FIRST SERIES.—Plants of *Helianthus annuus* were used as phytometers for this series, which was conducted at the Alpine Laboratory. On the basis of sunlight representing 100 per cent, light intensities

on a clear day in the shade habitat were as follows: 9 A.M. 0.992; 12 M. 1.125; and 3 P.M. 0.694 per cent. Air temperatures were approximately 6° higher in the sun habitat, the average day and night temperatures being 73° and 56° F. in the sun garden and 65° and 53° F. in the shade habitat. Soil temperatures taken from thermometers inclosed in the phytometers averaged 53° in the sun garden and 52° F. in the shade. The temperature of the garden soils was approximately 57° in the sun garden and 55° in the shade (table I).

TABLE I

FIRST EDAPHIC SERIES, JULY 10-AUGUST 3, 1929 (CF. FIG. 1)

	TRANSPIRATION PER 100 SQ. CM.														
	7/11	7/12	7/13	7/14	7/15	7/16	7/17	7/18	7/19	7/20	7/25	7/29	7/30	7/31	8/1
Sun.	19.0	19.0	23.0	14.0	10.0	7.4	9.0	14.0	5.2	12.0	51.0	54.0	9.6	5.1	3.7
Shade....	6.9	7.0	7.0	4.2	3.6	4.2	4.4	3.9	1.3	1.7	19.0	13.0	2.6	3.5	.9
	Average stem diameter (mm.)														
	8/2	8/5													
Sun.	4.4	4.5	4.6	4.6	4.9	4.9	4.8	5.9	6.6	7.1
Shade....	4.3	4.5	4.6	4.7	4.7	4.7	4.7	5.4	5.8	5.7
	Leaf area (sq. cm.)														
	8/2	8/5													
Sun.	142	161	174	187	201	215	216	244	334	380	493
Shade....	119	140	161	182	195	189	190	215	270	301	339

The experiment was started July 10 and ended August 3, 1929. Weighings were made at 5 P.M. daily from July 10 to 21, on July 26, and daily from July 30 to August 3. Leaf areas were taken at regular intervals. On August 3 the dry weight of the plants was determined.

Transpiration determined in three sun and shade habitats gave similar results (fig. 1).

SECOND SERIES.—Plants of two species, *Mertensia sibirica* and *Helianthus annuus*, were employed as phytometers for the final edaphic series. A slight rain of 0.02 inches was recorded during the experiment. Cloudy conditions prevailed during the afternoons of the nineteenth and twentieth, the mornings of these two days being clear or with intermittent clouds. The day of the twenty-first

was clear, while the morning of the twenty-second was intermittently cloudy. No leaf measurements were made, the water losses being determined on the bases of standardizations made before and after the series. Light readings were made in the shade area with a hand-photometer (table II).

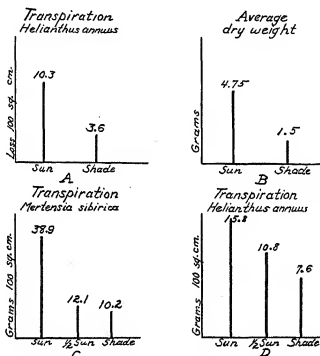


FIG. 1.—First series: A and B, July 10 to August 3, 1929 (cf. table I); C, July 5-7 1930; D, July 23-28, 1930.

TABLE II

LIGHT PERCENTAGES OBTAINED WITH HAND-PHOTOMETER

	8/19/31			8/20				8/21							
	9 A.M.	12 M.	3 P.M.	6 A.M.	8	10	12 M.	6 A.M.	8	9	10	12 M.	2 P.M.	4	6
Percent-age	2.2	17.5	2.7	0.3	1.3	3.3	20.0	1.1	1.1	1.6	1.8	20.0	8.0	4.1	0.6

Average light intensities, determined over a period of four hours, were approximately 0.91 cal./cm.²/min. in the sun and 0.025 cal./cm.²/min. in the shade. The maximum intensity was 1.40 in the sun and 0.084 cal./cm.²/min. in the shade, the minima being 0.382 and 0.012. Light intensities were measured by means of a pyranometer, which measures intensity of the total radiation falling

TABLE III
SECOND SERIES; TRANSPIRATIONAL AND INSTRUMENTAL DATA

	8/19						8/20						8/21						8/22					
	IO A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	8 A.M.	IO A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	8 A.M.	IO A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	8 A.M.	IO A.M.					
Transpiration, H. minus	14.8	10.0	7.9	7.3	1.7	6.7	11.0	13.3	7.0	5.7	4.7	4.3	14.0	18.3	14.0	14.0	8.5	7.8	10.0				
Transpiration, M. minus	13.3	10.2	7.1	1.8	4.0	5.8	11.5	13.8	4.3	4.3	3.3	4.3	13.3	16.0	10.0	12.4	6.21	5.8	7.5				
Transpiration, M. sibirica	6.0	4.5	1.0	1.0	3.0	4.0	4.0	2.0	4.5	5.3	1.0	5.0	8.0	3.0	2.5	2.5	3.5				
Outside temperature, 6 in. high	76	74	65	71	63	52	73	72	76	69	70	64	53	72	77	83	70	55	75	77				
Outside temperature, 6 in. high	Shade	63	66	64	58	49	59	66	70	60	63	59	51	62	69	72	72	70	64	54				
Soil temperature	Shade	62	74	72	60	57	61	68	72	72	65	59	50	58	62	72	70	59	52	54				
Soil temperature	Shade	68	84	84	78	73	59	65	75	73	73	73	59	58	62	73	80	81	81	84				
Soil temperature	Shade	74	71	65	62	52	54	56	57	58	58	52	52	54	57	61	63	54	56				
Humidity, psychrometer	Sun	50	45	59	41	59	49	37	41	29	31	37	45	49	33	20	44	17	46	32				
Humidity, psychrometer	Shade	52	46	66	67	75	58	36	54	46	69	80	68	57	55	43	31	47	78	68				
Saturation deficit	Shade	3.74	4.39	2.58	4.06	2.52	2.80	4.87	4.96	4.44	3.73	3.73	4.47	5.25	4.36	3.03	2.87	3.00	2.75	4.15				
Pitch evaporimeter, c. lost	Shade	3.3	2.9	2.58	3.99	2.47	2.80	4.96	5.05	4.53	3.83	3.83	4.57	5.39	4.58	3.26	3.00	3.00	2.75	4.15				
Evaporimeter, c. lost	Shade	0.6	0.8	0.4	0.7	1.5	1.7	1.3	1.8	0.9	0.9	1.0	1.2	1.2	1.4	1.9	1.6				
Evaporimeter, c. lost	Shade	0.08	0.07	0.08	0.05	0.01	0.09	0.06	0.05	0.18	0.03	0.10	0.15	0.07	0.02	0.30				
Evaporimeter, inches lost	Sun	0.08	0.07	0.08	0.05	0.01	0.09	0.06	0.05	0.18	0.03	0.10	0.15	0.07	0.02	0.30				
Evaporimeter, inches lost	Shade	0.02	0.02	0.01	0.00	0.03	0.01	0.01	0.01	0.00	0.01	0.03	0.03	0.01	0.04	0.03	0.04	0.09	0.04				
Aurometer, gain in miles	Shade	5.0	6.0	3.3	1.2	44.0	4.1	3.4	5.7	4.2	3.3	23.4	4.0	3.1	4.0	4.1	5.8	2.9	44.8				
Aurometer, gain in miles	Shade	0.0	0.4	0.4	0.1	0.8	0.8	1.5	0.6	0.0	2.5	0.4	0.5	0.5	0.5	1.1	0.1	2.1				

on a horizontal surface. Total water losses with plants of *Mertensia sibirica* as phytometers for the 4-hour period were 8.2 gm. in the sun and 1.6 gm. in the shade. Air temperatures varied from 62° to 76° in the sun and from 59° to 71° in the shade; while relative humidities averaged 49 and 52 per cent in the sun and shade respectively. These data indicate that radiant energy is an important factor in transpiration.

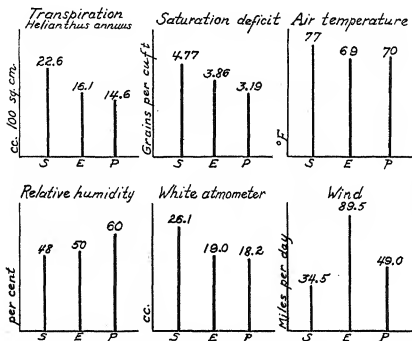


FIG. 2.—Third series, April 16-17, 1931: S, Santa Barbara; E, dunes exposed; P, dunes protected.

The principal experiment was started at 10 A.M., August 19 and closed at 10 A.M., August 22, 1931. Weighings and measurements were made every 2 hours from 6 A.M. to 6 P.M. (table III).

The stomatal conditions of the plants in the two habitats were practically similar.

SANTA BARBARA REGION

THIRD SERIES.—This experiment was conducted at three stations, one located in the coastal sagebrush association, and two in the sand dunes. One of the dunes stations was on an exposed knoll with a high wind velocity, while the second was in a protected area which was surrounded by *Lupinus chamissonis* and *Solanum douglasii*. The temperature, humidity, and saturation deficit data are based on

noon readings. The experiment was started at 6 A.M., April 16 and closed at 6 P.M., April 17, 1930 (fig. 2).

FOURTH SERIES.—This series was conducted during the week of March 2-9 at the Santa Barbara and the protected dunes stations. Plants of *Helianthus annuus* were employed as phytometers. Read-

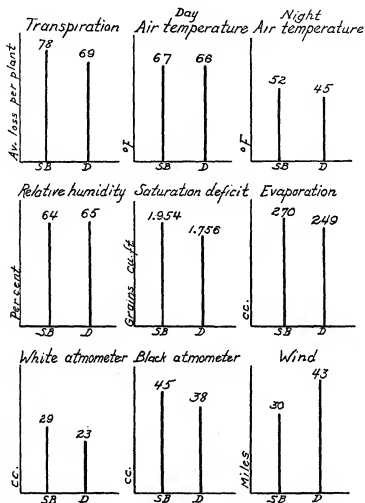


FIG. 3.—Fourth series, March 2-9, 1931

ings were made daily. During this period, water loss at the mountain station was approximately the same as at the Santa Barbara station (fig. 3).

FIFTH SERIES.—The third experiment at Santa Barbara was conducted April 7-10 at the mountain and the Santa Barbara stations. The mountain station was located at an elevation of 3000 feet. Plants of *Helianthus annuus* were used as phytometers. Weighings were made daily (fig. 4).

Discussion

PIKE'S PEAK REGION

Radiant energy seems to be the most important factor between sun and shade habitats. Other factors, such as air and soil tempera-

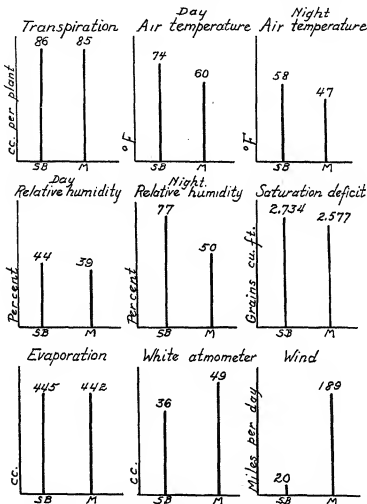


FIG. 4.—Fifth series, April 7-10, 1931: SB, Santa Barbara; M, mountain

tures, relative humidities, and saturation deficits, do not show the great differences that radiant energy does, and are probably therefore of less importance.

There were greater transpirational losses, growth, and dry weights in the sun than in the shade habitat. These edaphic studies also indicate a relationship between growth and transpiration.

There was a gradual decrease in transpiration from the beginning to the end of the experiment (table I). This is in agreement with

MAXIMOV's results (4). The results secured here with *Helianthus annuus* and *Mertensia sibirica* in edaphic conditions are in general accord with those of CLEMENTS and GOLDSMITH (2, 3).

The vegetation of sun and shade habitats varies according to the density of the shade. With lower light intensities, only tolerant species are able to grow. In the deeper shade of the montane zone most of the species are reduced in size, many not flowering; while in more moderate shade conditions the species are larger. The tolerant climatic species, for instance *Pseudotsuga mucronata*, are able to grow and reproduce in shade conditions, and consequently are to be found dominating the shady north slopes and ravines; while the dry south slopes are occupied by more intolerant species, for instance, *Pinus ponderosa*.

SANTA BARBARA REGION

Transpirational studies were conducted in the Santa Barbara region primarily to determine the relationships between the dune vegetation and that of the coastal chaparral.

Water losses were found to be lower in the dunes than in the coastal chaparral. Saturation deficits, air temperature, and evaporation were also greater in the coastal chaparral. Relative humidities and wind were higher in the dunes. These facts seem to indicate that the coastal sand dunes are not so xeric as is supposed. The probable explanation of the dune vegetation lies in the edaphic conditions rather than in the climatic, the presence of the sand being the important factor.

Transpiration, saturation deficit, evaporation, and wind were greater in the exposed than in the protected dune area. Relative humidity was higher in the latter.

In these experiments, as in the preceding ones, the ratios between transpiration and any one factor showed closer relationships with air temperature and relative humidity.

Experiments conducted in the coastal chaparral at elevations of 200 and 3000 feet showed transpiration at the two stations to be practically similar. Environmental factors at the two stations were variable. The losses from the white atmometer cup plainly show the effect of wind.

These results seem to indicate a definite correlation between factor, function, and vegetation. The sand dunes, although really more

mesic than the chaparral climatically, appear xeric, owing to the growth of the vegetation in a sandy soil. Throughout the chaparral association the total effect of all climatic and edaphic factors produces similar results in functional and vegetational responses, even though the individual factors vary.

Evaluation of the various factors

A second series of experiments was conducted to determine the chief external factor or factors influencing transpiration and the effect of each factor.

GENERAL.—Initial experiments were conducted in the alpine tundra at an elevation of 12,100 feet. Thirty sunflower plants were used in each of these experiments. The plants were potted in glasses, sealed, and placed in openings cut in the Celotex lids of boxes. The sunflowers were weighed hourly, and soil and air temperatures were recorded at each weighing.

The results indicated that a low soil temperature is an important factor in limiting transpiration, since a soil temperature of approximately 37° F., even with a rising air temperature, caused the plants to wilt. As the soil temperature rose, the transpirational curve seemed to follow more closely the air temperature curve. Later, however, when a cloud obscured the sun, the transpirational curve dropped more rapidly than did the air temperature, indicating that radiant energy is also an important factor in transpiration.

SOIL TEMPERATURES.—Experiments to determine the effect of various soil temperatures on transpiration were conducted at the Alpine Laboratory during the summers of 1930 and 1931.

Three conditions, cold, intermediate, and warm, with respective temperatures of 36°, 51°, and 113° F., were used for the initial experiment. Thirty standardized sunflower plants were divided into three groups of ten each, weighed and placed in the conditions already described. When reweighed, the batteries showed the following losses: cold, 19.0; intermediate, 46.2; and warm, 53.3 gm. The results show a rapid rise of the transpirational curve with a comparatively low increase of soil temperature, and a flattening off of the transpirational curve with high soil temperatures. These results indicate, as does the initial experiment conducted in the alpine tundra, that when soil temperatures get above 40° F., they are not as im-

portant in influencing transpiration as they are below this temperature.

This experiment was repeated and others conducted to check further the results obtained. The data for a typical experiment are shown in figure 5.

Four different soil temperature conditions: cold, two intermediate, and warm, were used. The cold temperatures were secured by packing the phytometers in a Celotex-lined box with ice, water, and

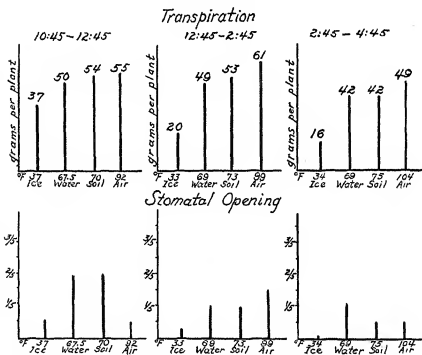


FIG. 5.—Graph of transpiration, soil temperature, and stomatal opening

salt. For the next higher soil temperature, the plants were placed in a box with water from a mountain stream running through it. The third condition was secured by placing the containers in a large box filled with soil. The plants were thus on a level with those in the other conditions. The highest soil temperature was secured by placing the phytometers on the surface of the soil, exposing them to the air temperature. Lids were constructed for each box in order to prevent a direct effect of the ice, water, and soil on the immediate environment of the various batteries. The sky conditions for this experiment were ideal, being practically cloudless.

The stomata of the plants of the various batteries were examined. Standardized plants of *Mertensia sibirica* were used as phytometers

in this experiment. The data shown on the graph are averages for the period.

These results further strengthen the previous conclusion as to the effect of soil temperature on transpiration and the flattening of the latter curve with higher soil temperatures. In addition there is seen to be a close correlation between stomatal opening and water loss. The plants in the ice-box began to wilt toward the end of the first period, and were all wilted by the middle of the second period. In the beginning the stomata of these plants were one-fifth open to closed. With the plants remaining in the wilted condition, the stomata became more tightly closed, and the losses consequently diminished. Further proof that low soil temperature is the predominant factor is derived from the fact that with the same stomatal opening, plants having soil temperatures of 37° and 92° F. gave losses of 37 and 55 gm. respectively (fig. 5). That a reduced stomatal opening limits transpiration is also shown by a comparison of the losses of the plants in the warm and intermediate conditions of the three periods. The relationship of soil temperature, stomatal opening, and transpiration is still further shown in the third period. In the first two periods the plants in the soil had been losing more than those in the water-box with similar stomatal openings. In the last period the stomata of the plants in the soil started to close before those of the plants in the water-box, consequently limiting the water losses, which were equal for the soil and water conditions for this period.

The plants were restandardized at the end of the experiment. The results indicate that the plants were not physiologically affected by the different conditions of the experiment.

RADIANT ENERGY.—Other experiments were conducted to determine the effect of radiant energy on transpiration. An equatorial mounting 5 feet tall with a cardboard top 3 feet square was constructed. The mounting was tall enough to permit free air movement, and could be adjusted to keep the plants always protected from the sun.

Standardized *Mertensia sibirica* plants were used for the experiment. Light readings were made with the pyranometer in the shadow cast by the mounting and also in the open. Air tempera-

tures, leaf temperatures, and relative humidity readings were also taken.

Leaf temperatures were approximately 3.5° F. higher in the open than under the mounting.

TABLE IV
RELATIONSHIP BETWEEN RADIANT ENERGY AND TRANSPIRATION

	AVERAGE H ₂ O LOSS PER PLANT (GM.)	AVERAGE LIGHT INTENSITY (CAL.)	AVERAGE AIR TEMPERATURE (° F.)	AVERAGE RELATIVE HUMIDITY (PER CENT)
Sun.....	24.7	0.911	70.4	49.6
Shade.....	9.7	0.096	70.2	49.0

TABLE V
EFFECT OF RADIANT ENERGY ON TRANSPIRATION

DATE (1931)	SPECIES	CONDITION	TIME	LOSS (GM.)	RADIANT ENERGY	TIME	LOSS (GM.)	RADIANT ENERGY CAL./ CMP./MIN.
8/13	<i>Mertensia sibirica</i>	{ Open	6-9	24.0	0.720	9-12	37.0	1.370
		{ Cheesecloth	"	21.0	0.280	"	31.0	0.610
		{ Muslin	"	17.0	0.150	"	27.0	0.340
8/14	<i>Mertensia sibirica</i>	{ Open	3-5	30.0	0.720
		{ Cheesecloth	"	21.0	0.250
		{ Muslin	"	19.0	0.180
8/17	<i>Mertensia sibirica</i>	{ Open	10-12	24.0	1.270	12-2	24.0	1.150
		{ Cheesecloth	"	20.4	0.478	"	14.8	0.452
		{ Muslin	"	17.4	0.288	"	11.0	0.244
	<i>Helianthus annuus</i>	{ Open	"	27.0	1.270	"	30.0	1.150
		{ Cheesecloth	"	21.0	0.478	"	15.0	0.452
		{ Muslin	"	20.0	0.288	"	10.4	0.244
8/18	<i>Helianthus annuus</i>	{ Open	"	20.0	1.120	"	20.0	1.420
		{ Cheesecloth	"	17.0	0.428	"	17.0	0.453
		{ Muslin	"	18.0	0.274	"	16.0	0.354

The results show a higher light intensity and a higher water loss in the sun than under the mounting. The ratios for radiant energy and water loss for the two conditions were 9.5 and 2.35 gm. respectively. The fact that the ratio for radiant energy is much higher than that for transpiration indicates that above a certain point radiant energy ceases to have much effect on transpiration.

As table IV shows, air temperature and humidities were similar in the two conditions, but leaf temperatures were slightly higher in the open.

The results of later experiments conducted with different light intensities indicated that radiant energy is one of the principal factors affecting transpiration.

SOIL MOISTURE.—In order to check the relation of different soil moistures to transpiration, sunflower plants were grown in a uniform soil and standardized. The plants were then divided into three groups, one set allowed to approach the wilting coefficient (or a low

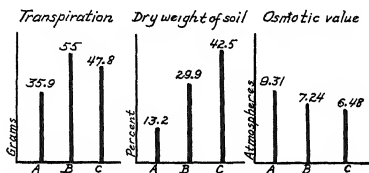


FIG. 6.—Graph of transpiration, percentage of dry weight of soil, and osmotic concentrations of the plant in atmospheres of pressure.

water content), another brought to a medium content, and the third given a high holdard. The experiment was conducted for a 5-day period, at the end of which time water contents were figured on the basis of dry weight and the osmotic values were determined.

Water loss apparently increases with the water content until the optimum conditions are reached. In this experiment the highest water content gave a water loss below that of the medium, owing undoubtedly to poor aeration. Osmotic values showed a constant decrease with increased soil moisture (fig. 6).

Summary

1. Radiant energy seems to be the most important factor between sun and shade habitats. Shade vegetation does not respond to any one factor, however, but rather to all of the environmental conditions.

2. Although individual factors varied, the environmental complex

at two different stations produced similar functional responses in the coastal chaparral association.

3. The xeric character of the vegetation of the sand dunes is due to an edaphic rather than to a climatic condition.

4. Soil temperature, below approximately $37-39^{\circ}$ F., is an important factor in limiting transpiration. Water loss seems to be increased by higher soil temperatures up to a certain point where the curve flattens off. The effect of different soil temperatures requires further study.

5. Transpiration was found to be highest in soils of medium water content, next in saturated soils, and lowest in soils with low water contents.

6. Osmotic values showed a constant decrease with increased soil moisture.

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CHEMICAL COMPOSITION OF *ASPERGILLUS* *NIGER* AS MODIFIED BY ZINC SULPHATE¹

NANDOR PORGES

Introduction

The ubiquitous black spore-bearing fungus, *Aspergillus niger*, produces a number of organic acids (1, 4, 9, 11) which vary with the methods of cultivation. Inoculated into a concentrated sugar medium kept at 30° C., the fungus produces considerable amounts of citric acid. The addition of ZnSO_4 to such a solution increases the yield of this acid per unit of sugar utilized (6). This salt has a marked effect on the type of growth. The surface is covered with a thick mycelial mat, heavily wrinkled and corrugated, dipping deeply into the liquid, thus permitting greater contact between the medium and the growth. The typical black spores are missing, having been replaced by spore bodies of an olive green to gray tinge. The addition of the ZnSO_4 increases the growth two- to threefold (8). On the other hand the growth on a solution containing no ZnSO_4 presents a different situation. The mycelium forms a thin pellicle, profusely covered with black spores, and the heavy folds and wrinkles are absent. Such marked differences in growth induced by the ZnSO_4 must be due to the differences in the chemical composition of the mycelium obtained from each treatment.

Experimentation

Sufficient mycelium was grown on solutions containing 10 per cent sugar and harvested. One liter of solution A contained: sugar, 100 gm.; NaNO_3 , 4.0 gm.; K_2HPO_4 , 1.0 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 gm.; FeCl_3 , 0.02 gm. Solution B had 0.1 gm. ZnSO_4 in addition.

The media were distributed to a depth of 4 to 5 cm. in Erlenmeyer flasks, and after sterilization were inoculated with a spore suspension of *Aspergillus niger* which was previously grown on CZAPEK's agar (3). After 7 days' incubation at 28° C. the pellicles were re-

¹ Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

moved, washed in three changes of distilled water, and dried at 70°–80° C. The growths were finely ground, and portions were taken for the determinations of moisture, nitrogen by Kjeldahl method, ash, carbon (2), and zinc. The amounts of titratable acidity, citric acid, and sugar were determined in the solution (6). These results have been presented in table I.

One can readily see that the presence of the zinc salt has more than doubled the yield of mycelium per unit of available sugar.

TABLE I
RESULTS OF GROWTH OF *ASPERGILLUS NIGER* IN 1 LITER OF
10 PER CENT SUCROSE MEDIUM

	SOLUTION	
	A	B
ZnSO ₄	Absent	0.100 gm.
Sugar available.....	100 gm.	100 gm.
Sugar utilized.....	49.5 gm.	77.9 gm.
Mycelium (dried at 70° C.).....	10.15 gm.	22.85 gm.
Moisture content (per cent).....	8.82	9.63
Dry mycelium (100°).....	9.26 gm.	20.65 gm.
Sugar utilized per gm. of mycelium..	5.34 gm.	3.77 gm.
Nitrogen available.....	0.6588 gm.	0.6588 gm.
Nitrogen in mycelium (per cent)....	3.12	2.61
Nitrogen in mycelium.....	0.2955 gm.	0.5392 gm.
Carbon in mycelium (per cent).....	45.82	45.58
Carbon in mycelium.....	4.3432 gm.	9.4119 gm.
C/N ratio.....	14.69	17.45
ZnSO ₄ recovered in mycelium.....	Trace	0.0403 gm.
Titratable acidity per liter, N/10...	2191 cc.	2487 cc.
Citric acid recovered per liter.....	8.65 gm.	12.16 gm.

Although the amounts of sugar and nitrogen utilized have increased, yet parts of these remained in solution. About 40 per cent of the zinc was found in the organism. There has been a high storage of energy material in the mycelium, as indicated by the carbon-nitrogen ratio, which has been widened from 14.69 to 17.45.

The two types of growth were then analyzed in more detail, following the procedure for the proximate analysis of organic matter advocated by WAKSMAN (10). The equivalent of 10 gm. of oven-dried material was carefully wrapped in filter paper and extracted with ether in Soxhlets for 16 hours in order to obtain the ether-soluble portion. The residue was transferred from the filter papers

into beakers and extracted with 95 per cent alcohol for one-half hour on the steam bath. This was filtered through a hardened filter paper which had previously been dried and weighed. An aliquot of the alcoholic extract was evaporated to dryness in a tared dish, dried, weighed, ignited, and weighed again in order to obtain the alcohol-soluble substances and the ash. Sugars, both total and reducing, and total nitrogen were determined in other portions of the extract.

The residue remaining after the alcohol treatment was removed from the paper and extracted with distilled water at room temperature for 24 hours. The solution was filtered through the same paper and soluble matter, ash, sugars, and nitrogen were determined in portions of the aqueous extract.

The residue left after this treatment was transferred into the original beaker and treated with hot water for one hour under flowing steam. This was filtered hot through the original paper. The residue and the paper were dried at 70° C. for several hours and then for three or four hours at 100° C. and weighed so as to obtain the weight of the residue insoluble in these various solvents. Aliquots of the solution were used to determine soluble matter, sugars, nitrogen, and ash.

The dried residue was extracted with 150 cc. of 2 per cent HCl for five hours under flowing steam. The hot extract was filtered through the original paper, washed free from acid, and the residue dried as previously to determine the weight. Reducing sugars and total nitrogen were determined on the combined filtrate and washings. The reducing sugar multiplied by 0.9 gave the hemicellulose content.

The residue, after ascertaining its weight, was carefully ground with a mortar and pestle. Two 1 gm. portions of the dried residue were treated with 10 cc. portion of 80 per cent H_2SO_4 for two hours. To each flask, 150 cc. of distilled water was then added and the flask with the contents autoclaved for one hour at 15 pounds' pressure. These extracts were filtered hot through small dried and tared filter papers, washed thoroughly with distilled water, dried (first at 70° and then at 100° C.), and weighed. Ash was determined on one of the residues by igniting it. Total nitrogen was determined on

the other residue. The weight of the residue minus the sum of weights of the ash and of the protein (nitrogen $\times 6.25$) gave the lignin in 1 gm. of material left after the dilute HCl treatment. It was necessary to multiply the weight of this lignin by the weight of the residue from the 2 per cent HCl extract to obtain the lignin content in the original 10 gm. of mycelium.

The solution and filtrate from the H_2SO_4 treatment were combined. Nitrogen and reducing sugars were determined in aliquots. The reducing sugars multiplied by 0.9 gave the cellulose content in 1 gm. of residue remaining after the 2 per cent HCl treatment. This value was multiplied by the weight of the material remaining after the dilute acid treatment to obtain the total cellulose content of the 10 gm. of mycelium.

Each analysis was run in duplicate on portions equivalent to 10 gm. of oven-dried material. The results of the detailed analyses have been compiled in table II, while in table III the data have been presented on percentage basis. This method of analysis gave a high degree of recovery and defined the differences in composition between the two types of growth.

The fractions differentiated by this method of procedure contained the organic compounds soluble in the solvents used and may be divided as follows (10):

1. In the *ether extract* are found the fats and oils, and also a part of the resins and waxes.
2. In the *alcohol extract* are found the waxes, resins, pigments, alkaloids, and some of the sugars.
3. In the *cold-water extract* are found the various sugars, amino acids, some proteins, various organic acids, and alcohols.
4. In the *hot-water extract* are found starches, pectins, tannins, proteins, amino acids, and other organic acids.
5. In the *dilute-acid extract* are found the carbohydrates insoluble in water but soluble in dilute acid. These are the hemicelluloses, including pentosans and hexosans, which account for the greater part of this extract, and starches. In addition there are present amino acids and proteins.
6. In the *80 per cent H_2SO_4 extract* are found those carbohydrates not soluble in dilute acids but soluble in concentrated acids and

TABLE II

PROXIMATE ANALYSIS OF GROWTH OF *ASPERGILLUS NIGER* (ON OVEN-
DRY BASIS) GROWN UPON 10 PER CENT SUCROSE MEDIA

	GRAMS	
	ZNSO ₄ ABSENT	ZNSO ₄ PRESENT (0.1 GM. PER LITER)
Weight of dry sample taken.....	10.00	10.00
Ether extract.....	0.269	0.518
Nitrogen.....	0.003	0.006
Alcohol extract.....	1.135	1.290
Reducing sugars.....	0.154	0.154
Total sugars.....	0.185	0.207
Nitrogen.....	0.022	0.027
Ash.....	0.004	0.007
Cold-water extract.....	0.523	0.884
Reducing sugars.....	0.045	0.042
Total sugars.....	0.137	0.225
Nitrogen.....	0.016	0.018
Ash.....	0.193	0.180
Hot-water extract.....	0.577	0.530
Reducing sugars.....	0.035	0.029
Total sugars.....	0.077	0.060
Nitrogen.....	0.023	0.016
Ash.....	0.027	0.044
Weight of residue.....	6.881	6.607
2 per cent HCl extract.....		
Reducing sugars.....	2.350	3.475
Nitrogen.....	0.104	0.075
Weight of residue*.....	4.082	2.729
80 per cent H ₂ SO ₄ extract.....		
Reducing sugars.....	0.303	0.453
Nitrogen.....	0.013	0.009
H ₂ SO ₄ residue.....	0.629	0.524
Nitrogen.....	0.026	0.031
Protein.....	0.163	0.194
Ash.....	0.001	0.003
Lignin (by difference).....	0.465	0.327

* Residues ground and 1.0 gm. samples taken for analysis.

which give glucose as the product of hydrolysis. These include the celluloses. Amino acids and proteins are also found in this extract.

TABLE III
PROXIMATE CHEMICAL COMPOSITION OF *ASPERGILLUS NIGER* GROWN
UPON 10 PER CENT SUCROSE MEDIA; PERCENTAGE
OF DRY MATERIAL

	PERCENTAGE	
	ZNSO ₄ ABSENT	ZNSO ₄ PRESENT (0.1 GM. PER LITER)
Ether-soluble fraction		
Fats, etc.....	2.50	4.80
Proteins.....	0.19	0.38
Alcohol-soluble fraction		
Waxes, sugars, etc.....	9.98	11.21
Proteins.....	1.37	1.69
Cold-water fraction		
Sugars.....	4.23	7.71
Proteins.....	1.00	1.13
Hot-water fraction		
Starches.....	4.34	4.30
Proteins.....	1.43	1.00
Dilute HCl fraction		
Starches, hemicelluloses, etc.....	20.25	31.28
Proteins.....	6.50	4.69
80 per cent H ₂ SO ₄ fraction		
Cellulose.....	11.15	11.12
Protein.....	3.32	4.69
Residual material		
Lignin.....	18.98	8.92
Protein.....	6.65	5.29
Ash in the fractions.....	2.25	2.34
Total accounted for.....	94.14	97.79
Protein accounted for in fractions.....	20.46	15.71
Total protein (N×6.25) by analysis.....	19.84	16.32
Total ash.....	2.76	2.88

7. The *residue* from the H₂SO₄ extract contains lignin-like products and proteins not acted upon by the various treatments. As mentioned previously, the amount of lignin-like material is ob-

tained by subtracting the weight of the protein and ash found from the weight of the residue.

8. The *ash* or inorganic fraction contains the various minerals which are available for the organism.

9. The *total protein* is calculated from the nitrogen determined by the Kjeldahl method. The nitrogen found in the various fractions should equal the total nitrogen.

Discussion

As can be noted from the tables, the addition of zinc induced marked changes. In the presence of this salt 77 per cent of the available sugar was utilized, while in its absence only 50 per cent disappeared from the solution as a result of the activities of the fungus. The metabolic processes were so modified that much less energy source (sugar) was necessary to obtain per unit of mycelium. Also the yield of citric acid was increased.

The proximate analysis of *Aspergillus niger* showed that the organism, under the influence of $ZnSO_4$, stored those organic substances within its cells that may be readily utilized. The ether-soluble substances, high in energy value, were almost doubled. The yield of this fraction was more than three times greater than that obtained in the absence of zinc per unit of sugar utilized: 63 gm. sugar gave 1 gm. of fatlike substances as compared with 198 gm. of sugar per gm. of ether-soluble fraction produced in the absence of zinc. A detailed analysis of the fats was not made, but variations may be expected, for PONTILLON (5) showed that the degree of acidity and the nitrogen source influenced the fat fraction of fungi. It is this fraction and the alcohol-soluble fraction that play an important part in the production of ergosterol (7).

The alcohol fraction showed differences in the coloring matter extracted; the heavy sporulated growth gave a dark black tinge, while the zinc-treated growth imparted a distinct green tinge.

The cold-water-soluble fraction showed a marked increase due to zinc and which was probably due to the various organic acids resulting from metabolic activities. Upon cooling the hot-water extract, a gelatinous precipitate settled out which was separated, washed, and dried. This was evidently a fungus starch, dextrin-like in character,

for the iodine test gave a red color and glucosazones were obtained upon hydrolysis.

The hemicelluloses obtained by the action of dilute HCl showed that the addition of the zinc salt increased these carbohydrates by 50 per cent. In this case, this easily available source of energy constituted the largest single group of substances present in the mycelium of the fungus grown in the presence of ZnSO_4 . The combination of this group with the nitrogenous group varied in the two fractions, as was indicated by the solubility of the proteins in dilute HCl.

The cellulose showed no differences in the two types of mycelium. On the other hand, the lignin-like substances were much less in the zinc-treated growth. This fraction is usually highest in plants that have gone through the vegetative stage and which are in the reproductive period of growth. The ratio of protein to lignin was much narrower in the growth obtained in the presence of zinc. In the insoluble material this ratio was 1.7 as compared with 2.8 in the mycelium grown on a zinc-free solution. One may gather that the lignin-like materials were formed at the expense of the hemicelluloses, for the sums of these two fractions were almost equal to each other in the two types of growth.

These analyses showed that the addition of zinc retarded the maturing of *Aspergillus niger*, holding it for longer periods in the vegetative stage and thus favoring the metabolic processes which act on and cause greater changes in the substrate.

Summary

1. *Aspergillus niger* was grown on a 10 per cent sugar nutrient solution in both the absence and presence of 0.01 per cent ZnSO_4 .
2. The addition of this salt repressed spore formation and favored the vegetative phase of growth. Such growth showed greater utilization of the available sugar, resulting in an increased yield of the dry mycelium and a greater production of citric acid.
3. The proximate analyses showed that zinc played an important part in the nutrition of the organism, resulting in marked differences in the composition of the mycelium.
4. The addition of ZnSO_4 to the solution increased those fractions of the mycelium readily available to the organism, namely, the

ether fraction, the cold-water-soluble fraction, and the hemicelluloses.

5. The other fractions, with the exception of the lignin-like material, remained constant.

6. The lignin-like substances were decreased by more than 56 per cent in the zinc-treated fungus. These substances appear to be formed at the expense of the hemicelluloses.

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PHASES OF THE ANATOMY OF ASPARAGUS OFFICINALIS¹

CHARLES H. BLASBERG

(WITH TEN FIGURES)

Introduction

Most of the early literature on asparagus is concerned with the application of fertilizer under field conditions, with the breeding of desirable strains, and with the improvement of cultural practices without regard to the details of structure and growth of the plant. Recently NIGHTINGALE and SCHERMERHORN (6) made a study of the nitrogen nutrition of the asparagus plant, and there are being conducted, during the progress of this work, further experiments on the mineral requirements, the record of which will appear later.

The present work was undertaken for the purpose of obtaining a more complete knowledge of the anatomy of the plant as a foundation for a better understanding of the mineral requirements, nutrition, and of experimental selection and breeding.

Materials and methods

Seeds of *Asparagus officinalis*, of the variety Carter's Special, were planted at weekly intervals in tubs in the greenhouse. Planting was begun on March 19, 1931 and continued until August 20, 1931, about 75 seeds being planted each week. These plants were used during the anatomical study. Also 1-year-old asparagus plants were grown in pots in sand culture. These plants were used for anatomical studies as well as for microchemical and macrochemical analysis. Further to supplement the material, 2-year-old and 12-year-old plants were dug from the field when needed.

For anatomical and microchemical studies, most sections were cut free-hand. When prepared material was required, older parts of the plant (after fixation) had to be treated with hydrofluoric acid in order to remove the calcium oxalate crystals and to soften the

¹ Journal Series paper of the N.J. Agricultural Experiment Station, Department of Vegetable Gardening.

tissue sufficiently to permit sectioning. The butyl alcohol method (10) was employed throughout for dehydration.

Anatomical study

GROWTH HABIT

Asparagus officinalis is a dioecious perennial which produces large edible spears. The mature aerial stems are much branched, and reach a height of from 1 to 2 meters. Subtending each branch is a parchment-like scale leaf; the characteristic phyllotaxy is a $2 \div 5$ arrangement. The small needle-like branches (phyllodes or cladophylls), which are the chief photosynthetic organs of the plant, occur in whorls at the nodes of the stem and branches (fig. 10).

The number of stems or spears produced by any given plant varies considerably. SCHERMERHORN (7) reported that many of the plants in his experimental plots had to be cut nearly every day during the cutting season. These plants produced 100 or more spears each. Other plants, which were receiving the same treatment, produced only four or five spears each during the cutting season. He concluded that the ability to produce spears is apparently inherent and is not entirely due to cultural practices or to the sex of the plant.

The root system consists largely of fleshy, unbranched storage roots which vary in thickness from $\frac{1}{8}$ to 6 mm. according to age, nutrient supply, and genetic composition. In general, plants which produce large spears have thick roots. The life of a storage root is not definitely known. JONES and ROBBINS (4) report a root which was at least 4 years old. Presumably the length of life depends upon nutrition and genetical composition. LOISEL (5) claims that the plant is constantly rejuvenated by the formation of new roots as the old ones die. As the storage roots die, the gradual decomposition of the inner cortical cells results in a condition of flaccidity. The stele and the suberized outer cortical cells remain intact for some time.

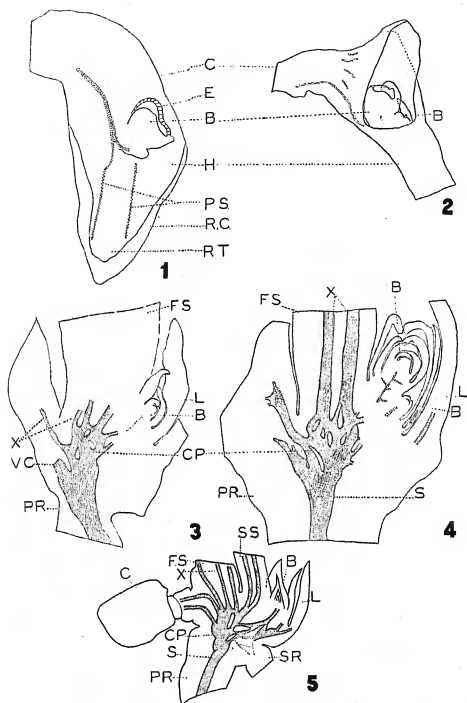
Since storage roots are produced so prolifically they soon form a thick mat in the soil, so that eventually any new roots which form must grow above the older ones. Also, as the crown continues its extension, it is mechanically inhibited by these roots from growing horizontally; consequently there is a gradual elevation of the crown until ultimately it reaches the surface of the soil.

The fibrous roots are the chief absorbing organs, and are the only part of an actively growing plant which contains nitrates (6), although under conditions which check vegetative growth, nitrates may be found elsewhere.

The rootstock, or crown as it is commonly called, is not a typical rhizome. It consists chiefly of the unelongated basal internodes of old stems. At the growing tip several large buds and many smaller ones can usually be distinguished. These become the aerial shoots which make their appearance above ground in a definite order, the bud nearest the last-formed spear being the next to elongate. The crowns of older plants exhibit a branching effect, for, as lateral buds develop, they extend the crown in new directions. There is a progressive development of new storage roots from the young tissue of the rootstock or crown. Most of these are ventral or lateral in origin, but occasionally one may originate from the dorsal side.

SEEDLING DEVELOPMENT

Seeds were germinated in the laboratory in moist petri dishes at 30° C., the optimum temperature for germination as determined by BORTHWICK (2). The hypocotyl emerged within 10 days after planting. Sections cut longitudinally through the hypocotyl showed the place of origin of the epicotyl (fig. 1). This first bud develops into the primary shoot (first spear), which usually attains a height of only 15 to 20 cm. and a diameter of about 2 mm. The second bud occurs in the axil of the basal scale leaf of the primary shoot, very slightly above the cotyledonary plate. Of the many seedlings sectioned and examined, none was observed in which the second bud originated below the cotyledonary plate as JONES and ROBBINS (4) have illustrated. This bud produces the second spear and, as shown in figure 3, it forms an angle of 180° with the cotyledon. At the base of this second spear, in some cases before it has elongated sufficiently to be called a spear, an axillary bud forms either to the right or to the left of the main growth axis; this bud produces the third spear (fig. 5). Another axillary bud may be formed at the base of the second spear but it lies in another plane (figs. 3-5). Ordinarily the crowns continue the elongation during the first year along the primary axis only, in which case these lateral buds remain dormant. If the bud



FIGS. 1-5.—Fig. 1, seedling 5 days after planting, showing origin of epicotyl: *e*, epidermis; *b*, axillary bud; *h*, hypocotyl; *ps*, procambial strands; *rt*, root tip; *rc*, root cap; *c*, cotyledon; X33. Fig. 2, seedling 10 days after planting; X25. Fig. 3, seedling 12 days after planting: *cp*, cotyledonary plate; *fs*, first spear; *l*, scale leaf; *x*, vascular bundles; *pr*, primary root; *vc*, vascular attachment to cotyledon; X25. Fig. 4, seedling 24 days after planting; X25. Fig. 5, seedling 33 days after planting: *sr*, secondary root; *s*, stele; *ss*, second spear; X10.

which forms the third spear originates to the right of the main growth axis, the bud which forms the fourth spear will be formed in the axil of a leaf to the left of the main growth axis. Thus there is an alternation of position which results in two rows of spear scars on the older rootstock. The sequential development of the buds on the rootstock forms an underground organ which is composed of many stem bases and is not a true rhizome like that of the canna.

ROOT STRUCTURE

STORAGE ROOTS.—New storage roots are formed at the base of the young, actively growing buds. They grow rapidly and may become 1 to 2 m. in length, depending upon the environmental conditions. Root hairs may be present. In the older parts of the root the epidermis, if still present, has become suberized. Although *Asparagus* is a monocotyledon, considerable increase in diameter of the storage roots is effected through cell division in the cortex. The number of cortical cells in a radial line between endodermis and epidermis varies from 35 to 50 in the older portions of the root, whereas the younger region contains only about 20 to 35 cells.

The cortex comprises by far the largest part of the storage root, the outer six to eight layers of cells being thick-walled and suberized (fig. 6). The remaining layers of cells are loosely fitted, having large intercellular spaces (fig. 6). These cells are the principal storage region of the plant. When 95 per cent alcohol is added to a section, a milky white precipitate forms which microchemical tests (3, 8) showed to be in part asparagose. Asparagose is an inulin-like substance which according to TANRET (9) yields upon hydrolysis 93 per cent fructose and 7 per cent glucose. It occurs within the cells and on the slide as minute lens-shaped crystals. The remainder of this milky fluid appeared to be chiefly a saponin. According to TUNMANN (8) the saponins are classified as glucosides, the constitution of which has not been definitely established; but they apparently yield a sapogenin together with hexosans, pentosans, or methylpentosans. When the plants are growing actively much fructose is present, but as they approach dormancy the content of fructose decreases and the sucrose content increases. Oil or fatlike globules (3) are present in all parts of the plant, but occur in greatest quantity

in the endosperm cells of the seed and in the cells of scale leaves surrounding young buds. Starch is not stored in quantity, but occasional starch grains may be found in the endodermis and innermost cortical cells. Bundles of needle-shaped crystals of calcium oxalate occur in parenchymatous cells in all organs of the plant, even in the fibrous roots. The endodermis (fig. 6) has deposits of suberin and later of lignin on its radial walls, whereas the thickening of the tangential walls consists chiefly of lignin.

In a young root the cells of the pericycle are small, angular, thin-walled, and closely packed. It is in this region that the secondary or fibrous roots originate. As the root reaches maturity, the walls of the pericycle cells increase in thickness.

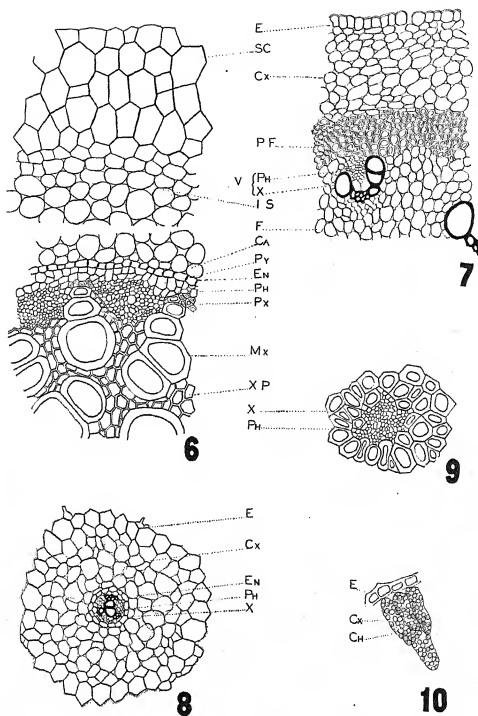
The xylem, which is all primary since there is no cambium, occupies the greatest portion of the stele. The protoxylem (fig. 6) is made up of small thick-walled cells with spiral thickenings. The number of protoxylem strands varies from 7 to 18. The metaxylem (fig. 6) has scalariform or reticulate thickenings. Each strand of xylem has one or more tracheae. The cells intervening between the xylem strands are thick-walled and lignified, so that in an old root there is a continuous cylinder of lignified tissue. Each phloem strand is composed of a few large sieve tubes and a relatively large number of small angular companion cells.

FIBROUS ROOTS.—In contrast to the storage roots, the fibrous roots are very small in diameter, usually 1 to 2 mm. The cortex is only three or five cell layers in thickness (fig. 8), and of these the outermost layer is usually suberized.

STEM STRUCTURE

The 1-year-old rootstock is usually unbranched, but dormant lateral buds are present which have the potential ability to extend the crown along a new growth axis. As the dormant bud develops to form an aerial shoot, at one of its basal nodes an axillary bud is differentiated. This axillary bud will form the next spear to either the right or the left of the axis of growth. Subsequent development is similar to the development along the primary axis.

Buds are differentiated very rapidly as the plant becomes older, so that a bud just before elongation has at its basal node another



FIGS. 6-10.—Fig. 6, fleshy storage root: *sc*, suberized cortex; *is*, intercellular spaces; *en*, endodermis; *ca*, casparian strips; *py*, pericycle region; *ph*, phloem; *px*, protoxylem; *mx*, metaxylem; *xp*, xylem parenchyma; $\times 138$. Fig. 7, cross-section through base of spear: *e*, epidermis; *cx*, cortex; *pf*, pericycle fibers; *v*, amphivasal vascular bundle; *x*, xylem; *f*, fundamental tissue; $\times 138$. Fig. 8, cross-sections of fibrous root: *e*, remains of epidermis; $\times 138$. Fig. 9, amphivasal bundle from crown; $\times 138$. Fig. 10, cells from phyllode: *ch*, chloroplast; $\times 200$.

bud which subsequently forms the next spear. This development is carried still further, so that the second bud has an axillary bud arising from it. This succession of buds gives the appearance of a cluster at the growth terminal of the crown. The number of buds is variable. Young seedlings planted March 18, 1931 had four buds in the cluster on August 25, 1931. One-year roots planted March 19 had nine buds in a cluster on August 10. The size of the bud immediately prior to its elongation is an index to the size of the spear. Large buds produce large spears and small buds, small spears. The buds just before elongation apparently contain the spear in miniature form. Longitudinal sections cut through young buds before elongation gave evidence of 17 to 20 scale leaves. Counts were made on the most luxuriantly growing stems of plants of about the same age, to determine the total number of scale leaves. The average was about 36 scale leaves per stem. Since the prepared sections showed leaves in only two of the five possible planes, the bud must have contained approximately the full number of leaves found on a mature stem. It seems logical to conclude, therefore, that at least most of the differentiation occurs prior to elongation. Elongation first begins in the lower portion of the stem, and continues upward so that the tip is still embryonic when the base is almost mature.

The epidermis of the stem contains only a few stomata and the guard cells of these contain chloroplasts. Chloroplasts are also present in the outermost cortical layers of the stem. Immediately within the cortex lies a cylinder of heavily lignified fibers, which contribute to the support of the erect stem. The vascular bundles are numerous and are scattered through the fundamental or ground tissue. The bundles are semiamphivasal, as shown in figure 7. The crown is stemlike in structure; and of the large vascular bundles which form a dense anastomosis, some are amphivasal (fig. 9) whereas others are semiamphivasal, as in the aerial portion of the stem.

The writer wishes to express grateful appreciation to Dr. RUTH M. ADDOMS and Dr. G. T. NIGHTINGALE for valuable suggestions and criticisms freely given during the progress of this work.

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COMPOSITION OF THE PULP AND SEEDS OF ADANSONIA DIGITATA

ROBERT A. GREENE

Introduction

Adansonia digitata, known as the baobab tree or "monkey bread" tree, is a native of the tropics. According to BAILEY (2) its average height is about 60 feet, but it sometimes attains a height of 90 feet, and it is said to have the thickest trunk of any tree in the world, the diameter sometimes exceeding 30 feet. The fruit, although variable in shape, is usually oblong, woody, indehiscent, and filled at maturity with a dry pulp in which there are many seeds. This pulp, known as "monkey bread," is used by natives as a food stuff. The fruit has a gourdlike structure, measuring 9 to 12 inches in length and approximately 4 inches in diameter.

This study was undertaken to determine the composition of the pulp and of the seeds. Although various investigators have discussed the uses of the fiber for various purposes, apparently the only study of the composition of the fruit has been made by PELLY (6).

Experimentation

The fruit was cut and the pulp and seeds separated from the remainder by means of a sieve. The seeds and pulp were then ground separately, and all determinations were made upon these samples. The relative weights of the shell, pulp, and seeds were approximately: shell, 45 per cent; pulp, 15 per cent; seeds, 40 per cent.

Both the seeds and the pulp were analyzed by the conventional methods for forage analysis, using the methods of the Association of Official Agricultural Chemists (1). The results are given in table I. These results show that the pulp contains a large percentage of carbohydrates, so the various carbohydrate fractions were determined, using the method of WAKSMAN and STEVENS (7), and the results are shown in table II.

PELLY found that most of the pulp was soluble in water, yielding

a mucilaginous liquid and an insoluble residue of cellular tissue. The aqueous extract possessed an acid taste and reaction. The acidity was attributed to free citric acid (which he identified), traces of malic acid, and acids of the pectic type, which probably occurred as acid potassium salts.

TABLE I
FEED ANALYSIS (AIR-DRY MATERIAL)

	PERCENTAGE	
	PULP	SEEDS
Moisture.....	7.65	8.48
Ash.....	6.36	3.73
Protein.....	5.94	11.56
Fat.....	0.24	6.80
Crude fiber.....	14.25	23.56
Nitrogen-free extract.....	65.56	45.87

TABLE II
PROXIMATE ANALYSIS OF PULP (MOISTURE-FREE BASIS)

	PERCENT- AGE
Ether-soluble organic matter.....	0.26
Alcohol-soluble organic matter.....	1.26
Cold water-soluble organic matter.....	50.96
Hot water-soluble organic matter.....	5.08
Hemicelluloses.....	8.25
Celluloses.....	9.44
Lignin.....	5.94
Crude protein.....	4.37
Ash.....	6.85
Total.....	92.41

Table II shows that approximately 50 per cent of the pulp is soluble in water. When water was first added to the material and mixed well, a gel was formed which was broken up by prolonged stirring. A highly colloidal solution resulted, which could be filtered only by suction. The filtrate was opalescent, resembling a suspension of starch. In order to determine the nature of this fraction, other determinations were made. The results are given in table III.

An examination of table I shows that the seeds contain 11.56 per cent of protein. A small portion was extracted with various solvents,

in order to determine the classes of proteins present. The method used was similar to that employed by JOHNSON (5) in his study of the chemistry of the tubercle bacilli. The results appear in table IV.

Discussion

Table I shows that the composition of the seed is somewhat similar to that of the seed of *Chenopodium album*, and also of a mixture

TABLE III
ANALYSIS OF COLD WATER-SOLUBLE FRACTION OF
PULP (MOISTURE-FREE BASIS)

	PERCENT- AGE
Reducing sugars (expressed as dextrose).....	13.18
Protein (N×6.25).....	2.01
Acid (expressed as citric).....	2.95
Ash.....	4.13
	<hr/> 22.27
Undetermined.....	28.69
Total.....	<hr/> 50.96

TABLE IV
DISTRIBUTION OF NITROGEN IN SEEDS

	PERCENTAGE OF TOTAL N
Water-soluble.....	16.19
Globulin (soluble in 10% NaCl).....	35.95
Glutelins (soluble in 0.2% NaOH).....	5.95
Prolamines (soluble in 70% C ₂ H ₅ OH).....	1.39
Other proteins (by difference).....	40.52
Total.....	<hr/> 100.00

of mesquite beans and pods, although the latter are somewhat lower in fat content (4). The composition of the pulp is similar to that of wheat bran (except that the protein and fat content of the baobab pulp are lower), dried beet pulp, and bread (4). Analyses of bread, however, show a lower ash and crude fiber content and a slightly higher protein content.

The seeds, so far as analysis shows, should make a very good stock food when ground. PELLY (6) states that the seeds contain no alkaloids or cyanogenetic glucosides, but that the seed coats are so

hard that the seeds must be boiled in order to render them soft enough to be used as cattle food.

The quantity of oil present is too small to give the seeds any commercial importance as a source of oil. There is, however, an interesting economic relation between the seeds of this tree and several species of *Dysdercus*, which are the principal cotton pests of the Sudan. These insects puncture the green bolls of the cotton plant, penetrating the seeds in order to feed upon the oil. When cotton is not available these insects feed, among other things, upon the oil in baobab seeds and in this way are able to survive until another cotton crop is available.

Tables II and III show that about 50 per cent of the pulp is soluble in cold water. In table III, about one-half of the water-soluble constituents are accounted for. The figures for reducing sugars appear high, and it is doubtful whether fresh pulp contains as great an amount. Qualitative tests for starch were negative, so the carbohydrates must be stored in some other form. It is possible that hydrolysis by enzymes or by the acid of the pulp may account for this apparently high value. Another possibility is the partial hydrolysis of some of the pectic substances. PELLY states that the pulp contains pectic acids which yield furfural and mucic acid, indicating the possibility of pentoses and galactose. Galactose is recognized as a constituent of pectins; but the production of furfural is not a specific test for pentoses, since galacturonic acid can be converted to arabinose under the conditions of the test. Although the percentage of pectic substances was not determined, it is probable that they would make up approximately one-half of the water-soluble fraction. GORTNER (3) gives the percentage of pectin in apple pomace as 15 to 18 (dry matter basis), lemon pulp 30 to 35, orange pulp 30 to 40, and beet pulp 25 to 30. It does not seem unreasonable to expect the pulp of *Adansonia digitata* to contain about 20 per cent of pectic material.

The water-soluble fraction contained 0.3 per cent of nitrogen, which is approximately 32 per cent of the total nitrogen. Tests for albumin were negative, which is to be expected since plant albumins seldom occur. Although a part of this nitrogen is probably in inorganic forms, it is possible that a large part occurs in organic forms.

The figures for citric acid are lower than those given by PELLY, who found 4.4 per cent. This amount, he claimed, is of no commercial value because of the interference of pectic substances with the extraction.

Table IV shows that 10 per cent NaCl extracted the major protein fraction. It is probable then that a globulin is the principal protein of the seed. Tests for albumin which were made on the water extract gave negative results.

There seem to be no commercial uses for either the pulp or the seeds. PELLY suggested that the seeds might be used as a manure or as cattle food. He also stated that in some parts of East Africa the pulp is used to coagulate Ceara rubber latex, a property due to its acid content.

Summary

1. The pulp and seeds of *Adansonia digitata* have been analyzed, and the results agree with previous analyses of PELLY.
2. A feed analysis shows that the seeds have a composition similar to the seeds of *Chenopodium album* or a mixture of mesquite beans and pods.
3. About 50 per cent of the pulp is soluble in water. Reducing sugars, acids, proteins, and ash comprise about one-half of this amount; the remainder is probably chiefly pectic substances. Neither the pulp nor the seeds gave tests for starch.
4. The principal protein of the seeds is a globulin, which apparently has never been isolated before.
5. The tree has an economic significance since it furnishes food to carry several species of *Dysdercus* (cotton pests) through periods when cotton seeds are not available.

The writer is indebted to Dr. R. H. FORBES, of Tucson, Arizona, who suggested this problem, supplied the material, and offered many valuable suggestions.

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BRIEFER ARTICLES

A DEMONSTRATION OF BASAL GROWTH¹

(WITH ONE FIGURE)

The gladiolus has been found very suitable to demonstrate basal growth of leaves. As lecture material the results present a clear-cut illustration of the origin of growth. In the laboratory this plant is excel-

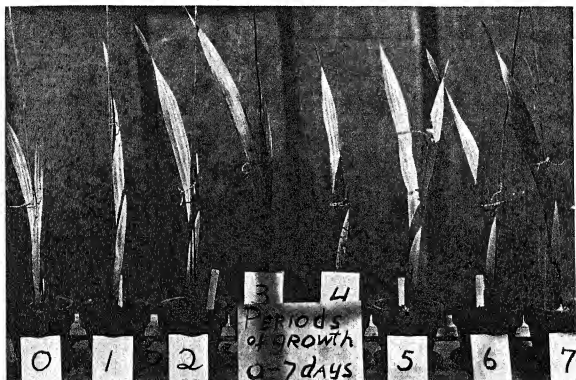


FIG. 1.—Basal leaf growth in gladiolus

lent for showing the effect of various factors on growth. An excellent contrast of old and new growth may be secured by dusting the plant with Nicotine Carrier dust. This application is a substitute for the tedious process of marking the leaf with India ink. Talcum powder may be used, either dusted or rubbed on, if the Nicotine dust is not available.

The dusting may be done when the plant has entered a vigorous growing stage. In a few hours it will be noted that new growth has broken the

¹ Approved for publication by the Head of the Department of Botany.

continuity of the dusted area, the break occurring at the point where the leaf emerges from its sheath. Figure 1 illustrates such a demonstration, showing the amount of growth in periods ranging from one to seven days. It also shows the increase in the rate of growth in the second leaf. This is particularly noticeable in the 3- and 5-day periods of the illustration.

For laboratory work the plants may be exposed to high and low temperature environments, and the growth areas measured at intervals. Table I gives the figures on the length of growth areas of plants kept for one week in cool and warm greenhouses.

TABLE I
GROWTH (IN CM.) OF GLADIOLUS LEAVES IN COOL
AND WARM AIR

PLANT NO.		FIRST LEAF (CM.)	SECOND LEAF (CM.)
Cool air	1.....	9.0	12.5
	2.....	8.5	16.4
Warm air	3.....	19.2	27.0
	4.....	21.5	26.0

This plant furnishes suitable material for the collection of data on basal growth under various conditions of time, light, and temperature. The gladiolus is not easily injured by rough handling and a sample may be used several times.

Corms harvested in the autumn will be ready for planting by February 1. If it is desired to make this demonstration in the autumn, corms should be placed in cold storage at about 35° F. by April 1. The corm germinates well if the lower half is buried in soil in a 4-inch pot.—C. J. GILGUT AND LINUS H. JONES, *Department of Botany, Massachusetts State College, Amherst, Massachusetts.*

CURRENT LITERATURE

ANNOUNCEMENT

Beginning with the current number, the *BOTANICAL GAZETTE* will be issued quarterly rather than eight times a year as formerly. The numbers will appear in September, December, March, and June.

This change will provide approximately the same number of pages per volume and at the same time will permit a saving in manufacturing costs. The adjustment also has enabled the publishers to reduce the amount of the annual subscription price, which is now \$8.

BOOK REVIEWS

Soil conditions and plant growth

The development of soil science continues its rapid pace under the stimulus of international congresses and societies for its promotion. The most valuable English summary of the soil in its relations to plant growth during the last 20 years has been RUSSELL'S¹ excellent monograph, which has been deservedly popular throughout the long period since the first edition appeared. The sixth edition of this famous work has just come from the press.

The appearance of the new edition was delayed by RUSSELL'S study of soil technology among his Russian colleagues. The task of sorting out the most valuable new knowledge gained from these contacts, and rewriting the work to incorporate the advances of the last five years, has been a difficult one; but with the aid of the Rothamsted staff it seems to have been accomplished in admirable manner.

The book is increased about 20 per cent in size. The number of chapters and general arrangement remain the same, but chapters IV, V, and IX have been given new titles. These are now listed as: IV, The soil in nature; (1) Changes in its mineral composition; V, The soil in nature; (2) Changes in the organic matter; and IX, Methods of characterizing soils. In the preceding edition these were: Chemical and physical relationships of the soil, Carbon and nitrogen cycles of the soil, and Methods of soil examination, respectively. The main additions are made in chapter II, on soil conditions affecting plant growth; chapter III, on the composition of the soil; and chapter IV. All chapters have been increased in size except the introduction, which has been changed but slightly, and the final chapter, which has been reduced to half its former size.

¹ RUSSELL, SIR E. JOHN, *Soil conditions and plant growth*. 6th ed. 8vo. pp. viii+636. Longmans, Green and Co. New York and London. 1932.

The work maintains its high position as the leading exposition of the soil as a medium of plant growth. The technicalities of soil science are subordinated to an understanding of the plant in its relation to the soil. This emphasis upon the reactions of crop plants to the soil environment is what has made it so useful and so popular. It has always merited the favor it has enjoyed among students of plant behavior.—C. A. SHULL.

Phylogeny of conifers

The first part of a very important contribution to the historical development of Coniferales and Cordaitales has been published by FLORIN.²

The author first raises the question about morphological and anatomical characters which can be studied in the fossil material of Coniferales and Cordaitales. From there he proceeds to a detailed comparative anatomical investigation of recent conifers and discusses their applicability to fossil species. This volume is primarily devoted to the treatment of the recent forms from this point of view. The most important organ for species determination which can be examined in fossils and which, therefore, is extensively discussed in recent forms, is the structure of the epidermis of the assimilatory organs. The stomata are treated in detail for all living families of conifers. This investigation is followed by a study of the variability of the epidermis structure of fully developed assimilatory organs. He uses these characters to explain the relation between the different genera of living conifers and finally he compares the stomata of the conifers with those of the other gymnosperms. Toward the end of the volume the importance of the examination of the cuticle for the investigation of fossil plants is discussed.

FLORIN's publication represents the first paper in a series of investigations in which comparative anatomical studies of the phylogeny of Coniferales and Cordaitales are to be carried out. The author extends his studies also upon the cuticles and stomata of other gymnosperm groups, as Ginkgoales, Cycadales, and Gnetales. This book is a step forward in our knowledge of recent, as well as fossil, gymnosperms, and the succeeding volumes should be looked for with great interest.—A. C. NOÉ.

² FLORIN, RUDOLF, Untersuchungen zur Stammengeschichte der Coniferales und Cordaitales. Erster Teil. Kungl. Svenska Vetenskapsakademiens Handlingar Tredje Serien. 8vo. 10: No. 1. pp. 588. Figs. 111; pls. 58. 1931.

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EFFECTS OF ULTRAVIOLET RADIATION UPON REPRESENTATIVE SPECIES OF *FUSARIUM*¹

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(WITH PLATE I AND SIX FIGURES)

Introduction

One of the most common fungi with which the market pathologist must deal is the ubiquitous *Fusarium*. There are very few vegetables on which *Fusarium* does not occur, either as a primary or secondary incitant of decay, and so the problem of identification of *Fusarium* species is an ever present one. During the course of recent work with species of *Fusarium* isolated from decayed onion bulbs, one of the chief difficulties has been the production in artificial culture of sufficient normal macrospores for taxonomic studies. Various methods of inducing sporulation have been tried, but in many cases these were unsuccessful or only inconsistently fruitful. Often microspores were produced in abundance but the macrospores, which are usually necessary for identification, were lacking. The consistent success of RAMSEY and BAILEY (14) in producing macrospores of the onion parasite *Fusarium cepae* Hanz. emend. LINK and BAILEY (8) upon irradiation with ultraviolet suggested the use of this method for unidentified strains of *Fusarium* from onion. In not all instances was the irradiation as beneficial as in the case of *F. cepae*, but in a large percentage of strains it was helpful and in some it induced marked increase in spore production.

¹ Contribution from the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Department of Botany, University of Chicago, cooperating.

Because of the value of ultraviolet radiation in the work on the taxonomy of onion Fusaria, irradiation of authentic species of *Fusarium* from the various sections of the genus was tried, in the hope that this might prove helpful in the ever difficult problem of taxonomic diagnosis of the many species within the form genus. Investigations on the effect of ultraviolet irradiation upon members of the genus *Fusarium* have been made by others, but most of the work has dealt with the lethal action of the rays. SIBILIA (18) describes the lethal effect of ultraviolet on two species of *Fusarium*, *F. echinosporum* and *F. fuliginosporum*, first described by him (17) but not mentioned by WOLLENWEBER in the *Fusaria Autographice Delineata* (29, 30). SIBILIA found that exposure of the conidia of these two strains in drops of distilled water or of 1 per cent glucose solution at a distance of 12 cm. from the unscreened quartz mercury vapor lamp for two minutes or over invariably resulted in death. The age of the conidia seemed to play no part in their resistance to ultraviolet. Chlamydospores were more resistant than conidia. In most of the experiments, exposures as short as one second, even at 90 cm. from the arc, resulted in death; however a distance of 2 meters permitted germination of the spores, the air screening out the more detrimental rays. The contents of spores which were killed by radiations were greatly plasmolyzed, and showed numerous small globular masses or, rarely, a single ellipsoidal mass. Conidia germinating under irradiation had abnormal germ tubes, often contorted and soon arrested in growth.

FULTON and COBLENTZ (5), working with *Fusarium* sp. from orange fruit, found that the spores, in water suspensions, were killed by 1-minute exposures 6 inches from an unscreened 110-volt quartz mercury burner having a mercury cathode and a tungsten anode, operated on 320 watts (80 volts, 4 amperes). Mycelium from germinated spores was more easily killed than were ungerminated spores of the various fungi tested. The fungicidal effectiveness of treatment with ultraviolet depended on the intensity of the radiation and on the wave lengths of the component rays; the shorter the wave length the more lethal the effect with a given intensity. The killing effect was cumulative, several short intermittent exposures (rest periods were the same length as exposure periods) having almost the

effect of one continuous exposure of the same total time. The killing effect increased with the lengthening of the time of action, and also with the increase in intensity of irradiation.

DILLON WESTON and HALNAN (4) also worked on *Fusarium* sp. and found that the mycelium was not killed but that growth was inhibited. Their tests were not run with an unscreened lamp, however, but with vitaglass and Sanalux petri dish covers transmitting only 50 to 70 per cent of the ultraviolet light from a quartz mercury-vapor lamp (Hanovia artificial alpine Sun, 2.5 amp. 210 v. direct current type). Potato agar plates were inoculated from a pure culture of the *Fusarium*, a needle being used to carry the spores and mycelium into the medium. Irradiation was commenced the same day and was continued for a fortnight with daily 9-minute exposures. They found that the mycelium of *Mucor* and *Fusarium* grew farther down into the medium in the irradiated plates; "consequently, aerial development is retarded and the sporangiophores and conidiophores appear stunted." After the first week of irradiation the plates were reversed and the unirradiated controls were subjected to a week's treatment while the previously treated plates were held as controls. "The effect of this treatment was to inhibit the growth of the original controls and to encourage growth in the culture that had originally been retarded by the action of the light." They suggest that in many cases the mycelium may not be killed but rendered dormant for some considerable time.

STEVENS (21), in a group of fungi with which he says he obtained no interesting results from irradiations of 10, 30, and 50 seconds on corn meal agar "except stunting of colony and perhaps darkening and distortion or suppression of spore structures," lists: *Fusarium batatatis*, *F. conglutinans*, *F. niveum*, *F. oxysporum* (faint reddening on irradiation), *F. vasinfectum*, and *Fusarium* spp. (many). He also irradiated *Gibberella saubinetii*, which has as a conidial stage *F. graminearum*, and obtained with irradiations of 30 and 50 seconds distinct reddening of the mycelial tip, although he does not report the production of perithecia as in the case of *Colletotrichum lagenarium* (25).

JOHNSON (7) found that the aerial mycelium of *F. batatatis* Wr. collapsed, and that there was an increase in pigment within 24

hours after a 10-minute irradiation with Schumann rays. The collapsed mycelium "revealed a very granular protoplasm and the granules were relatively large." The mycelium was not entirely killed by a 25-minute exposure. Both macrospores and microspores occurred on controls but were more abundant after irradiation. Eight-day old cultures were less susceptible than those 5 days old. He found, also, that the ultraviolet transmitted by a Hanovia quartz mercury-arc lamp through cellophane (5790-2201 A.u.) produced no lethal effects on spores newly planted on agar, even if the dosage was as much as 50 minutes at 8 inches from the lamp. Three to 4-day old cultures given five daily exposures of 5-10 minutes through cellophane at 8-16 inches from the arc showed a "pronounced increase in pigment and a very noticeable suppression of growth." Zonation of the colony also occurred. The same types of reactions occurred to a less degree with filters transmitting to 2302 and 2536 A.u. With filters transmitting no lower than 2907 A.u. no variation was present after exposures as long as 3 hours at 8 inches.

RABINOVITZ-SERENI (13) worked with *Fusarium martii* and *F. poae*, and found that the spores of neither species were very resistant to the action of the ultraviolet emitted by a Gallois di Lione quartz mercury-vapor lamp at 20-cm. distance. Only a small percentage of those irradiated for 12 minutes or more germinated, and the germ tubes of these were abnormal.

Although much attention has been centered on the fungicidal action of short waves, some work has been published on the stimulating action of ultraviolet rays on fungi. NADSON and PHILIPPOV (9) reported a stimulating effect of ultraviolet rays on the growth of *Saccharomyces cerevisiae* and *Mucor genevensis* Lendn. In a subsequent paper (10) they reported work on four species of yeast and on *M. guilliermondii* Nad. & Phil. The fungi were exposed in petri dishes covered with a screen of thick glass or metal, in the center of which was a small round opening; the lamp used was a Bach quartz mercury-vapor model (100-120 volts, 3.8-4 amp.) transmitting from 2200 A.u. to 5700 A.u. Exposures of 10-20 minutes at 30 cm. from the arc were made 24 hours after inoculation of the plates with water suspensions of the organisms, and several days later it was found

that the part of the cultures submitted to direct radiation was dead, a sterile circle corresponding to the opening in the center of the screen. The rest of the colony, protected by the screen, continued to develop normally except in a narrow zone surrounding the sterile area, in which zone the yeast, influenced by the oblique rays, showed an intense multiplication and growth. Abnormal budding also occurred. In this "zone of stimulation" *M. guilliermondii* produced sporangia in great numbers although zygosporoes were lacking. In *M. genevensis*, on the other hand, it was the sexual reproduction which was induced in this area, the increased number of zygosporoes forming a black border to the sterile zone.

PORTER and BOCKSTAHLER (11) exposed *Colletotrichum lindemuthianum* and *Cephalothecium roseum* to wave lengths of 3630-3650 A.u. transmitted through a filter by a Cooper-Hewitt quartz mercury-arc lamp 60 cm. distant. They report "inhibition of growth and collapse and probably death of the aerial hyphae," and say: "In addition to these effects spore formation is inhibited sharply in *Cephalothecium* but on the other hand slightly accelerated in the case of *Colletotrichum*."

In the case of *Colletotrichum phomoides*, HUTCHINSON and ASHTON (6) were able to accelerate sporulation and retard growth by exposure of 3-day old cultures 12 cm. from the full mercury arc (Cooper-Hewitt) for 15 seconds to 2 minutes. Acervuli formed 24 hours after irradiation, with maximum development taking place in cultures irradiated for 30 seconds to 1 minute. Similar effects could be obtained by irradiating spores previous to planting on agar plates.

STEVENS, in works quoted and in subsequent papers (22, 23, 24), reports that he was able to induce pycnidia formation in such forms as *Camarosporium* sp. and in some strains of *Coniothyrium*, and to produce perithecia and acervuli in some strains of *Glomerella cingulata*. Doses approximately 300 times longer were required to kill conidia than were needed to induce perithecia of the last-named fungus. There was no such regularity in the induction of acervuli of *Glomerella cingulata* as in the case of perithecia. Both acervuli and perithecia failed to appear on irradiation of some strains.

DILLON WESTON (3) found that the retardation of growth result-

ing from exposure to ultraviolet wave lengths shorter than 400 m μ "may result subsequently in an acceleration of the development of the organism." Cultures of *Fusarium culmorum* were given one irradiation for different periods with light from a Hanovia sun-lamp. Although the primary reaction was one of retardation, subsequently the irradiated cultures sporulated abundantly whereas the non-irradiated cultures sporulated sparsely. In correlating these two facts he states, "it is assumed here that the initial retardation forces the organism to an earlier maturity."

The present paper attempts to make a comparative study of the effects of irradiation upon sporulation and upon certain physiological responses, such as color production and growth rate, in representative species from the described sections of the form genus *Fusarium*. All the sections are represented except section II, Macroconia, section IV, Submicrocera, and section V, Pseudomicrocera, of which no authentic cultures were obtained. All subsections are represented except Aquaeductuum under section Eupionnotes, which contains those species formerly placed in section Camptospora Wr. (29). The systematic arrangement followed is that given by WOLLENWEBER in his revised Delineata (30) and in the *Fusarium* monograph (32).

An attempt has been made to select, so far as possible, species which were typical for each section. Tests were made also with some of those having known perfect stages, in order to see whether irradiation would serve to stimulate production of the perithecial stage. More species were chosen from section Elegans (28, p. 28; 31, p. 741) than from any other section, because this group contains more of the important plant parasites and because there is often greater difficulty in producing macrospores, and in distinguishing some of these forms, than is the case with species from the other sections.

Most of the cultures used were obtained from Dr. H. W. WOLLENWEBER or from Dr. O. A. REINKING at the time of the *Fusarium* conference in Madison, or are cultures brought to the conference by the writer and used in the studies (34) there. Credit is given in a footnote connected with table I for authentic cultures obtained from other sources.

Methods

Since, in the experiments with *F. cepae* and *Macrosporium* (14), irradiation through vitaglass covers transmitting to 2650 A.u. had proved most satisfactory, this glass was employed in most of the present experiments. Five-inch vitaglass squares were fitted as covers by molding paraffin rings about the edges of petri-dish bases. The lower half of the petri dish was held tightly against the vitaglass square until the paraffin had set, and then was slipped out. A little vaseline was mixed with the paraffin to make it less brittle when cold. These covers were sterilized with alcohol, and when dry were used to replace the ordinary petri-dish lid over the fungus culture previous to its exposure to ultraviolet.

In some of the experiments, a Stewart health-lamp equipped with therapeutic "A" carbons was used and operated on alternating current at 12 amperes through 110-volts resistance. This lamp is equipped with a Corex D filter which transmits traces between 2700 and 2800 A.u., feebly between 2800 and 2850 A.u., and absorbs none of the wave lengths above 2880 A.u. When the filter is not used the lamp gives some feeble transmission as low as 2300 A.u. Increased sporulation may be obtained with the Corex D filter, but in order to get the full effect of the lower wave lengths transmitted by the vitaglass it was necessary to remove the filter from the lamp. With the filter removed the heat produced tended to melt the paraffin rings if the plates were exposed for a long time at a distance of 40 cm. from the arc. For this reason most of the comparative studies were made with a Cooper-Hewitt quartz mercury-arc lamp, which does not produce so much heat. The mercury lamp was operated on alternating current at 4 amperes through resistance from a 110-volt line.

Plantings were made on potato dextrose agar, which had been found to be a very suitable medium for all species tested. For the purpose of these experiments it made little difference whether the inoculum used was a small piece of mycelium or a spore dilution, providing the same type of inoculum was used for control and test plates. The fungi were allowed to grow for four days before irradiation was begun. Some trials were made in which irradiation was begun on the second day, but the results were never so satisfactory.

If the cultures were much older than four days at the beginning of the tests, the mycelium often filled the plates before the end of the tests, thus hindering the measurement of growth rate.

Except in the experiments with various filters and in some of the direct exposures attempting to produce the sexual stage, the exposures were made through vitaglass covers 40 cm. from the unscreened mercury arc. Unless otherwise stated, all cultures were exposed for 15 minutes for each of three successive days. In all tests several plates were used for each exposure, and the average reading of many mounts made for total number of microspores and macrospores present, the results being recorded in terms of macrospore percentage of the total. Controls were held for an equal length of time in the diffused light of the laboratory, and the exposed plates were held in the same place as the controls when not actually under the lamp. All experiments were duplicated and questionable ones checked several times.

Results of irradiation through vitaglass

That not all species of *Fusarium* responded to irradiation with increased macrospore production, as did *Fusarium cepae*, is apparent from table I, which gives a summary of some of the experiments in which the cultures were irradiated through vitaglass for 15 minutes on each of three successive days. In most of the species exposed to ultraviolet there was an increase in total sporulation, if not in the percentage of macrospores produced. In some species, as *F. orthoceras* App. and Wr. var. *albidoviolaceum* (Dasz.) Wr. (Section XIV), the irradiated plates, although showing very few macrospores (fig. 1 E), actually had such an increase in microspore production as to produce an exposed pionnotes or at least marked zonation of the colony (fig. 1 A), while the control plates showed only aerial microspores and non-zonate mycelium. On the other hand, in forms like *F. stilboides* var. *minus* Wr. (Section XII), where microspores are usually lacking or scarce and where the percentage of macrospores present is therefore high in the control plates, the amount of macrospores in the irradiated plates was often increased to form sporodochia (fig. 1 B) or a definite exposed pionnotes, as contrasted with few spores present in the controls. Usually increase in sporula-

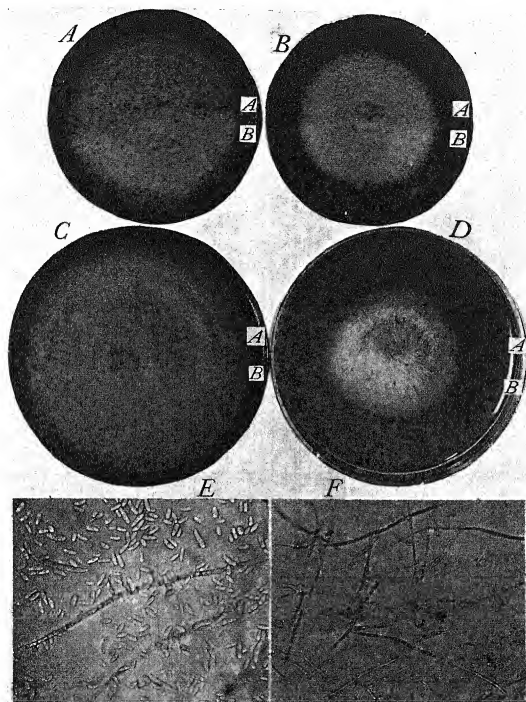


FIG. 1.—A-D, petri-dish cultures of *Fusarium* spp. showing effects of three daily 15-minute exposures to ultraviolet irradiation through vitaglass (2650-6690 A.u.), 40 cm. from quartz mercury arc: A, *F. orthoceras* var. *albidoviolaceum*: (a) irradiated portion showing zonate growth and deeper color due to sporulation; (b) control half (covered with photographer's black paper during irradiation); B, *F. stilboides* var. *minus*: (a) irradiated portion showing sporodochia; (b) control half; C, *F. nivale*: (a) irradiated portion showing more powdery appearance and zonation; (b) control half; D, *F. dimerum*: (a) irradiated portion showing exposed pionnotes; (b) control portion; E, *F. orthoceras* var. *albidoviolaceum* showing scarcity of macrospores after three daily 15-minute exposures through vitaglass; F, *F. argillaceum* showing injured spores and mycelium and chlamydospores produced on old portion of colony which had received three daily 15-minute exposures through vitaglass.

TABLE I

SUMMARY OF EXPERIMENTS SHOWING EFFECTS OF THREE SUCCESSIVE 15-MINUTE EXPOSURES TO ULTRAVIOLET TRANSMITTED THROUGH VITAGLASS (LOWER LIMIT OF TRANSMISSION, 2650 Å.U.)

SECTION	SPECIES OF FUSARIUM	PERCENTAGE MACROSPORES		OTHER DIFFERENCES
		IRRADIATED	CONTROL	
1. Eupionnotes	<i>F. dimerum</i> Penz. (R)*	0.0	0.0	More microspores and deeper color on exposed plates
	<i>F. merismoides</i> Cda. var. <i>majus</i> Wr. (Syn. <i>F. betae</i> (Desm.) Sacc. (W))	10.9	7.7	Total sporulation (macro- and microspores) greater on tests
2. Macroconia	No representative
3. Spicarioides	<i>F. decemcellulare</i> Brick. (R)	1.0	—1.0	More microspores on exposed
4. Submicrocera	No representative
5. Pseudomicrocera	No representative
6. Arachnites	<i>F. nivale</i> (Fr.) Ces. (D-L)	68.5	53.5	Exposed culture more powdery
7. Sporotrichiella	<i>F. sporotrichioides</i> Sherb. (B)	60.7	32.8	Thicker pionnotes on exposed plates
8. Roseum	<i>F. anguoides</i> Sherb. (R)	6.0	—1.0
9. Arthrosporiella	<i>F. semitectum</i> B. & Rav. (W) <i>F. semitectum</i> var. <i>majus</i> Wr. (R) (Syn. <i>F. incarnatum</i> (Rob) Sacc.)	23.7 94.3	19.4 64.1	Thicker pionnotes on exposed plates

* Letters in italics refer to source of the culture as follows:

B., W. H. W. WOLLENWEBER
C., C. O. A. REIDING
D., J. A. S. DIER through G. K. K. LINK
E., W. W. GILBERT
F., L. L. HARTER

J., HELEN JOHANN
K., O. A. REIDING
S., M. SIAPOVALOV
T., N. J. J. TAUBENHAUS through A. G. NEWHALL

W., H. W. WOLLENWEBER
W.-J., WOLLENWEBER through HELEN JOHANN
*W*_{Wis}, Wisconsin laboratory
W-L, Wisconsin laboratory through G. K. K. LINK

TABLE I—Continued

10. Gibbosum	<i>F. equiseti</i> (Cda) Sacc. f. 1 <i>Wr.</i> (R) (Syn. <i>F. ossiculum</i> (B & C) Sacc.) <i>F. scirpi</i> L. & <i>F. var. acuminatum</i> (E. & E.) <i>Wr.</i> (R) <i>F. scirpi</i> L. & <i>F. var. longipes</i> (Wr. & Rg.) <i>Wr.</i> (R) ? <i>F. scirpi</i> L. & <i>F. var. compactum</i> Wr. (B) ? <i>F. scirpi</i> L. & <i>F. var. compactum</i> Wr. 2nd test after spores were once ob- tained by irradiation	37.7	27.0 50.0 38.1 0.0 69.0	Exposed pionnotes on irradiated plates Very few spores in control; more plen- tiful on exposed } See text Total sporulation greater after irradi- ation
11. Discolor ss. Neesiola ss. Trichothecioides	<i>F. heterosporum</i> Nees. var. <i>paspali-</i> cola (P. Henn.) <i>Wr.</i> (W) <i>F. trichothecioides</i> <i>Wr.</i> (B)	1.8	0 to -0.5	Total sporulation greater after irradi- ation A good many 2-septate spores on ir- radiated plates
ss. Saubinetii	<i>F. macroceras</i> <i>Wr.</i> & Rg. (R) <i>F. culmorum</i> (W. G. Sm.) Sacc. (W-J) <i>F. sambucinum</i> Fuckel (S)	-0.5 83.2 93.8	-0.5 0.0 91.0	Total sporulation greater on exposed plates No microspores on controls Irradiated plates and controls look alike
12. Lateritium	<i>F. sambucinum</i> Fuckel (B) <i>F. stilboides</i> var. <i>minus</i> <i>Wr.</i> (R) (Syn. <i>F. fructigenum</i> Fr. var. <i>minus</i> <i>Wr.</i> f. 1 <i>Wr.</i> & Rg.)	95.0	0.0	No microspores on controls; few spores on treated plates Spores rare in control; plentiful in definite pionnotes on exposed plates

TABLE I—Continued

SECTION	SPECIES OF FUSARIUM	PERCENTAGE MACROSPORES		OTHER DIFFERENCES
		IRRADIATED	CONTROL	
13. Liseola	<i>F. neoceras</i> Wr. & Rg. (R)	35.2	3.7	Greater sporulation on exposed plates
	<i>F. moniliforme</i> Sheld. (R)	-1.0	0	" " " " "
	<i>F. "</i> " var. <i>subglutinaus</i> Wr. & Rg. (R)	6.0	3.2	" " " " "
	<i>F. moniliforme</i> Sheld. var. <i>erumpens</i> Wr. & Rg. (R)	-1	-0.5	" " " " "
	<i>F. orthoceras</i> App. & Wr. var. <i>longius</i> (Sherb) Wr. (W)	4.4	3	Exposed pionnotes and many microspores on irradiated plates; no pionnotes and few microspores on controls
14. Elegans ss. Orthocera	<i>F. orthoceras</i> var. <i>albidoviolaceum</i> (Dasz) Wr. (C)	-1	-0.5	Total sporulation greater on irradiated plates
	<i>F. orthoceras</i> var. <i>albidoviolaceum</i> (Syn. <i>F. asclerotium</i>) (strain from W)	1.7	0	Thin pionnotes on exposed plates; no pionnotes and few microspores on controls
	<i>F. orthoceras</i> var. <i>triseptatum</i> Wr. (R)	-1	-1	Exposed culture more powdery
	<i>F. bostrycoideus</i> Wr. & Rg. (R)	-0.5	-0.5	Total sporulation greater on irradiated plates
	<i>F. conglutinans</i> Wr. (Wis)	-1.0	0.0	Exposed pionnotes on irradiated plates; not on controls
ss. Constrictum	<i>F. "</i> " var. <i>callistephi</i> Beach (C)	7.4	-0.5	Thicker pionnotes and deeper color than controls
	<i>F. bulbigenum</i> Cke. et Mass. f. 1 Wr. (Syn. <i>F. lycopersici</i>) (W)	-1	0	As above; strains from 2 other sources acted the same
	<i>F. bulbigenum</i> Cke. et Mass. f. 1 Wr. (Syn. <i>F. lycopersici</i>) (strain 2-W)	4.3	3.7	Relatively few spores in controls; many in treated plates
	<i>F. bulbigenum</i> var. <i>batatas</i> Wr. (Syn. <i>F. batatatis</i> Wr.) (H)	4.8	0.8	

TABLE I—Continued

ss. Oxyposium	F. oxyposium Schlect. (R) " " (2nd strain S)	- I 2.3	-0.5 -0.5	Total sporulation greater on irradiated plates As above
	F. " " f. 1 Wr. (W)	2.2	0	Thinner pionnotes on controls; 2 strains from Harter acted same way
	F. (Syn. F. euoxysporum Wr.)	2	-1	No pionnotes on control; thin one on irradiated plates
	F. oxyposium f. 2 Wr. (S) (Syn. F. hyperoxysporum Wr.)	Strain A, 8 Strain B, 1	0	Pionnotes thinner and covered with mycelium in controls
	F. oxyposium f. 3 Wr. (Syn. F. cubense E.F.S.) (2 strains R)	69	28.3	Total sporulation greater on exposed plates
	F. oxyposium f. 5 Wr. (R) (Syn. F. oxyposium var. nicotianae Johns)	7.6	0	Exposed pionnotes on irradiated plates
	F. oxyposium var. aurantiacum (Lk.) Wr. (Syn. F. aurantiacum) (W)	51.0	5.0	Total sporulation greater
	F. cepae Hanz. emend. Link & Bail.			More mycelium in controls
	F. vasinfectum Atk. (G)	1	1	No aerial mycelium on exposed plates
	F. " " f. 1 Wr. (Syn. F. vas. var. inodoratum) (W)	96.9	83	Pionnotes thicker on exposed plates
	F. vasinfectum var. lutulatum (Sherb.) Wr. (R)	55.5	42.4	" " " " " "
	F. vasinfectum var. zonatum (Sh) Wr. (Syn. F. zonatum) (W)	28	4.9	" " " " " "
	F. vasinfectum var. zonatum f. 1 (Lk. & Bail.) Wr. (B)	81.9	69	" " " " " "
	F. vasinfectum var. zonatum f. 2 (Lk. & Bail.) Wr. (B)	59.0	51.2	" " " " " "
	F. redolens Wr. (W)	83.9	53.4	" " " " " "
15. Martiella	F. coeruleum (Lib) Sacc. (S)	96.8	0	No spores of any kind on controls; pionnotes on irradiated plates
	F. solani App. & Wr. var. medium Wr. (Syn. F. mali Taub.) (T-N)	59.1	3.4	Exposed pionnotes on irradiated plates
	F. javanicum Koord. (K)	39.9	17.0	See text
16. Ventricosum	F. argillaceum (Fr.) Sacc. (W-I)	73.2	65.9	Exposed plates show appressed moist mycelium, much stunting and many chlamydospores

tion was noticeable macroscopically in production of sporodochia or pionnotes, or at least in greater intensities of color in mycelium, stroma, and spore mass. In *F. nivale* (Fr.) Ces. (Section VI), although the increase in macrospore percentage was not marked, the treated colonies were much more powdery owing to the increased sporulation (fig. 1 C). In such species as *F. dimerum* Penz. (Section I), where macrospores do not occur, an increase in the microspore production resulted in an exposed pionnotes on irradiated plates (fig. 1 D), but there was no evident increase in septate over continuous spores. This increase was accompanied by an intensification of the mycelium color. RABINOVITZ-SERENI (12) reports such an intensification of colors in cultures of *F. poae* exposed to ordinary blue light, but does not report any associated increase in quantity of spores present.

In some species the macrospore percentage in exposed plates was more than double that in the control plates. In *F. neoceras* Wr. and Rg. (Section XIII) there was an increase from 3.7 to 35.2 per cent; *F. cepae* (Section XIV) increased from 5 to 51 per cent macrospores; and *F. solani* App. and Wr. var. *medium* Wr. (Syn. *F. mali* Taub.) (Section XV), which had given very few normal macrospores in ordinary laboratory culture, showed an average of 59.1 per cent macrospores after irradiating for 15 minutes through vitaglass on three successive days. In most species the response to irradiation was relatively consistent although the percentages varied. For instance, it will be noted (table I) that the average percentage of macrospores present in the *F. sporotrichioides* Sherb. (Section VII) plates after irradiation was 60.7, not quite double that in the control plates, whereas in the experiments summarized in table III the average macrospore production under vitaglass was 51.7 per cent, or five times that of the controls; but in all experiments there was a decided increase in percentage of macrospores. The average percentage for all the experiments with *F. vasinfectum* Atk. var. *zonatum* (Sh.) Wr. (Section XIV), when treated to three successive 15-minute exposures under vitaglass, was 12.1 as against an average of 3 per cent in the controls. Individual tests varied from 8 per cent in the irradiated plates with 0.5 per cent in the controls to 46.7 per cent in the irradiated plates with 5 per cent in the controls. Out of

ten such experiments with this species, each involving several plates, the percentage on the irradiated plates was usually five or six times that from the controls, was never less than three times, and was only once as high as ten times that obtained in the controls.

On the other hand, in an equal number of experiments with *F. conglutinans* Wr. and with *F. bulbigenum* Cke. et Mass. forma I Wr. (syn. *F. lycopersici* [Sacc.] Wr.) and others, the percentage was never increased by as much as 1 per cent under this treatment. The tendency to show definite, unmistakable macrospore production when exposed to the ultraviolet rays transmitted by a quartz mercury arc through vitaglass seemed to be characteristically present or absent for any one of the species tried in these experiments with but few exceptions. The exceptions were *F. scirpi* L. and *F. var. acuminatum* (E. and E.) Wr. (Section X), *F. javanicum* Koord. (Section XV), and *F. argillaceum* (Fr.) Sacc. (Section XVI).

In *F. scirpi* var. *acuminatum* most of the experiments showed striking increase in macrospore content after irradiation, but in two experiments the increase was slight, and in one the controls averaged a higher percentage than the treated plates. In this latter case, however, the number of spores actually present on the control plates was so small as to require examination of a great many mounts before sufficient spores were obtained for counting, whereas in the exposed plates the spores were plentiful although low in macrospore count. Where spores are rare, one would expect a high macrospore count with *F. scirpi* var. *acuminatum*, for even when not in "hochkultur" it produces few microspores. However, the macrospores in control cultures made from subcultures of a strain which had been carried long in artificial culture were often small 3-4 septate spores as contrasted with the well shaped normal 5-septate spores of the irradiated cultures.

F. javanicum is another species which ordinarily produces very few microspores, but the strain which had been received from REINKING in 1924 had been carried in artificial culture since that time and was producing a high percentage of 1-septate and small spores. In some tests the irradiated plates, in contrast to the controls (fig. 2 A), produced a fair percentage of normal 4-5-septate spores (fig. 2 B) but in other experiments irradiation had little effect. Apparent-

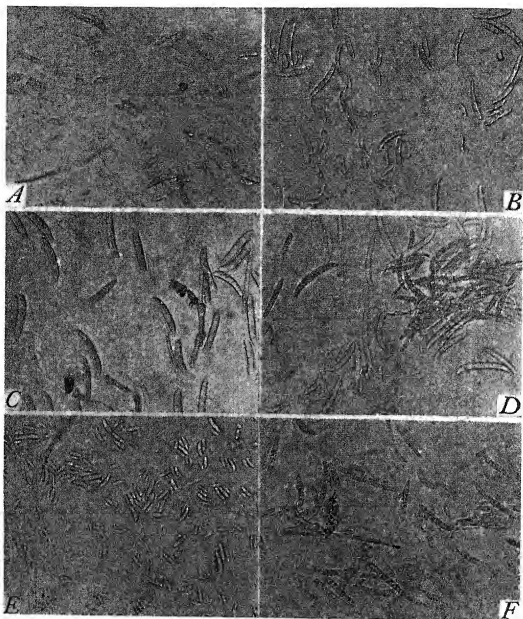


FIG. 2.—Effects of ultraviolet irradiation on cultures of *Fusarium* spp., placed 40 cm. from quartz mercury arc: A, *F. javanicum* control showing predominance of microspores; B, *F. javanicum* irradiated through vitaglass for three daily 15-minute periods with resultant increase in macrospore percentage; C, macrospores of *F. coerulescens* var. *zonatum*: D, abundance of macrospores produced as result of three daily 15-minute irradiations through Corning Corex A filter 980 A (2300–6690 A.u.); E, control showing scarcity of macrospores; F, plasmolysis of protoplasm in spores as result of three daily 15-minute exposures direct to the lamp (2230–6690 A.u.).

ly macrospore production by this method cannot be predicted for *F. javanicum*.

In the case of *F. argillaceum*, the macrospore percentage on the irradiated plates was usually a little higher than on the control plates, but sometimes this did not hold true. It was found that if the mounts were made only from the new growth that occurred during the 24 hours following irradiation, the macrospore percentage was always higher and the spores normal; but if the mounts were made only from the portion of the colony that had actually been irradiated, there was considerable variation in results. The spores (fig. 1 *F*) present in this irradiated area were usually abnormal in shape, contained refractive particles apparently in part fat and glycogen, or were plasmolyzed or breaking down, so that even though present they could not be used for study of normal spores. In all experiments great care was taken to secure uniform spore mounts from all over the colony, but in the case of *F. argillaceum* this was always more difficult, owing to the toughness and irregular growth of the stunted colony after irradiation. Even those rays transmitted by vitaglass usually resulted in disruption of spores and mycelium of *F. argillaceum*, and in retarded, abnormal growth. Lengthening the period of exposure to one half-hour resulted generally in such colony stunting (measured by radial increase and amount of aerial mycelium) and death of spores that recovery did not take place in the 24 hours succeeding exposure, with the result that the macrospore percentage was less than that of the controls at the close of the experiments. Shortening exposure to one 15-minute period gave better production of macrospores. The irradiation usually resulted in the production of many chlamydospores, whereas the control plates had very few; with longer exposures the growth often was largely chlamydospores.

The most outstanding usefulness of irradiation with ultraviolet is in inducing the production of spores by strains which have not been sporulating; and the method is an advantageous one, for if the species is one responsive to ultraviolet treatment macrospores are readily obtained in a very short time. Even the production of microspores is extremely helpful in such studies, for purification of the

culture by obtaining single spore strains and for help with taxonomic identification. One strain (tentatively placed in *F. scirpi* L. and *F. var. compactum* Wr. of Section Gibbosum), isolated from decayed onion bulbs, had never produced spores in artificial culture until irradiated, when normal spores suitable for taxonomic study appeared on the experimental plates. While the treated plates had many spores (75 per cent macrospores) the control plates were still without spores of any kind. In subsequent experiments with this fungus, when the inoculum was taken from the pionnotal stage which now occurred in subcultures made from the previously treated plates, irradiation failed to bring the percentage of macrospores noticeably higher than the controls which were already producing abundant macrospores, as shown in table I.

A culture of *F. coeruleum* (Lib.) Sacc., which likewise had failed to produce spores of any kind in artificial culture, was induced to produce abundant macrospores (fig. 2 C) with irradiation, while the controls remained sterile. A strain of *F. sambucinum* Fuckel, which was in "abkultur," was made to produce macrospores by irradiation although the controls produced no spores; and similarly, irradiated plates of *F. culmorum* (W. G. Sm.) Sacc. produced 83.2 per cent macrospores when no conidia could be found in the control plates. In the case of the latter species it was a matter of hastened spore production, for a week later both control and treated plates showed spores. Yet even after this interval of time effects of irradiation were evident, for the control plates averaged from 40 to 56 per cent macrospores and the exposed plates from 65 to 70 per cent. DILLON WESTON (3) also found hastened sporulation in irradiated cultures of this species, but the increase in macrospore percentage here given would seem to indicate that the effect of irradiation is something more than earlier maturation. Preliminary observations of irradiated *Fusarium* spp. indicate that changes in the composition of the mycelium may be associated with the assumption of sporulation following irradiation.

It is interesting to note in table I, and in subsequent tables, that the species which responded to treatment with marked increase in macrospore production are the saprophytes and decay-producing organisms and not the vascular parasites. The only vascular para-

site which showed appreciable increase was *F. oxysporum* Schlect. forma 5 Wr. (syn. *F. oxysporum* Schlect. var. *nicotianae* Johns.), whose control plates averaged 28.3 per cent macrospores while the irradiated plates averaged 69 per cent. This culture of *F. oxysporum* f. 5 is a subculture of Dr. REINKING's culture no. R119 of *F. oxysporum* var. *nicotianae*, which is the strain represented by WOLLENWEBER in Delineata 1012 (1930 Supplement) (30) and described by REINKING and WOLLENWEBER in Tropical Fusaria. In the latter reference (15, p. 192) the authors state in text and footnote:

Fusarium oxysporum var. *nicotianae* is the cause of a tobacco wilt in the United States, but the pathogenicity of the soil fungus described here has not yet been proved. . . . Under such circumstances the fungus described here, as isolated from Honduras soil and identified as *F. oxysporum* var. *nicotianae* may touch the border line or may be even a widespread saprophytic soil form of the banana wilt *Fusarium*, *F. cubense*.

Incidentally the strains here tested of the banana parasite, *F. cubense* E. F. S., also obtained from REINKING, gave little response to ultraviolet. Whether or not this variation in response of two large groups of *Fusarium* species will prove constant in tests with other species of these groups, and whether such differences will prove significant in separating vascular parasites from non-vascular strains, awaits further experimentation. It is possible that more response to light might be expected from organisms which were more often exposed to light in nature on the surface of decaying material, etc., than from the vascular parasites which are spread through the soil, less exposed to sunlight, and also less dependent upon spores for their spread. At least, in contrast with the response of the vascular parasite group, irradiation was most positive in its effect on the production of macrospores in the following sections: Sporotrichiella, Gibbosum, Discolor subsection Saubinetii, Martiella, and Elegans subsection Oxysporum (except vascular parasites).

It was found, as in the previous work with *F. cepae* (14), that irradiation of responsive species on several days in succession was better than one longer exposure. One day's exposure with responsive species gave results, but not so many macrospores developed as were obtained by several irradiations. Fifteen minutes under vitaglass on each of three successive days was a good average period for

this purpose; under some of the filters transmitting shorter wave lengths a shorter period of time was favorable. Increasing the exposure beyond 15 minutes sometimes resulted in a still greater increase in sporulation, sometimes not. Plates of *F. decemcellulare* Brick., given three 30-minute exposures, produced 3.1 per cent macrospores as against 1 per cent after three successive 15-minute exposures and in the controls; with half-hour exposures of *F. heterosporum* Nees. var. *paspalicola* P. Henn. it was possible to get 4.5 per cent as against 1.8 per cent under 15-minute treatments. But these differences are slight and it was usually found that if treatment for 15 minutes under vitaglass was not effective, longer exposures were seldom warranted, and in some fungi, such as *F. argillaceum*, were actually lethal or detrimental to normal vegetative and reproductive development.

Multiplying the numbers of days of exposure was often more effective for conidial production than lengthening the period of a single exposure; but in species entirely unresponsive to three 15-minute treatments, even this increase in number of irradiations failed to produce spores. Table II shows the results of three and of six daily 15-minute exposures with several species of *Fusarium*. In all of the experiments shown in this table several plantings were made to the plate, and the readings made on the seventh day were from different colonies on the same plates as those read for the fourth-day results. The readings on the fourth day were, of course, made before irradiation on that day. In a few species, such as *F. vasinfectum* var. *zonatum* forma 2 and *F. oxysporum* Schlecht. forma I Wr. (syn. *F. oxysporum* var. *euoxysporum* Wr.), there was noticeable increase in the six exposures, but in some of them (*F. javanicum* and *F. argillaceum*, for instance) the spore count was less at the end of the six days.

An attempt was made to see whether a cumulative effect could be obtained in some of the species which had failed to respond with macrospore production. For the purposes of this study, spores from plates which had already been irradiated were used as inoculum for new petri-dish cultures to be irradiated. The tests with *F. orthoceras* App. & Wr. will serve to illustrate the results. A strain which had been producing few spores in the stock tubes was used in the first experiments, and the usual tests involving three 15-minute

exposures resulted in a deepening of the color of the colony and the production of more microspores than the controls. Less than 1 per cent macrospores were present in controls and test plates alike. A duplicate experiment was run with three half-hour exposures which

TABLE II
EFFECTS ON SPORULATION OF FUSARIUM SPECIES OF THREE AND OF SIX
DAILY 15-MINUTE EXPOSURES TO ULTRAVIOLET RAYS
TRANSMITTED THROUGH VITAGLASS

SPECIES OF FUSARIUM	EXPOSED (PERCENTAGE MACROSPORES)		CONTROL (PERCENTAGE MACROSPORES)	
	3 DAYS	6 DAYS	3 DAYS	6 DAYS
<i>F. argillaceum</i> (Fr.) Sacc. (<i>W-J</i>)*	67.3	42.7	62.6
<i>F. bulbigenum</i> Cke. & Mass. f. 1 <i>Wr. (C)</i>	5.4	7.0	6.7	6.0
<i>F. conglutinans</i> <i>Wr. (W)s</i>).....	0 to			
" " var. <i>callistephi</i> Beach (<i>C</i>)	-0.5	3.9	0.0	2.0
<i>F. decemcellulare</i> Brick. (<i>R</i>).....	7.4	3.8	1.0	1.0
<i>F. heterosporum</i> Nees. var. <i>paspalicola</i> (P. Henn.) <i>Wr. (W)</i>	2.2	3.1	0.6	1.0
<i>F. javanicum</i> Koord. (<i>R</i>).....	2.0	4.5	-0.5	-0.5
<i>F. macroceras</i> <i>Wr. & Rg. (R)</i>	39.9	9.2	17.0	17.0
<i>F. moniliforme</i> Sh. var. <i>maius</i> <i>Wr. & Rg. (R)</i> ...	-0.5	-0.5	-0.5	-0.5
<i>F. orthoceras</i> App. & <i>Wr. (W)</i>	0.6	1.6	1.0	1.0
<i>F. " var. albidoviolaceum</i> (Dasz) <i>Wr. (W)</i>	1.0	1.0	-0.5	-0.5
<i>F. oxysporum</i> Schlecht. (<i>R</i>).....	1.7	0.9	0.0	0.0
<i>F. " forma 1</i> <i>Wr. (W)</i>	-1.0	-1.0	-0.5	-0.5
<i>F. " " 2</i> <i>Wr. (S)</i>	2.2	12.7	0.0	0.0
<i>F. " " 3</i> <i>Wr. (R)</i>	3.0	1.9	-1.0	1.0
<i>F. " var. aurantiacum</i> (Lk) <i>Wr. (W)</i>	-1.0	-1.0	0.0	0.0
<i>F. redolens</i> <i>Wr. (W)</i>	7.6	7.0	0.0	-0.5
<i>F. scirpi</i> L. & <i>F. var. acuminatum</i> (E. & E.) <i>Wr. (R)</i>	83.9	86.3	53.4	55.0
<i>F. semitectum</i> B. & Rav. (<i>W</i>).....	64.7	99.3	78.8	79.6
<i>F. vasinfectum</i> Atk. (<i>B</i>).....	28.9	22.1	18.5	16.6
<i>F. " strain 2</i> (<i>G</i>).....	2.0	4.8	3.8	3.5
<i>F. " forma 1</i> <i>Wr. (W)</i>	1.0	1.6	1.0	1.0
<i>F. " v. zonatum</i> (Sh) <i>Wr. f. 2</i> Lk. & Bail. (<i>B</i>).....	96.9	98.2	83.0	81.0
	56.8	78.6	55.0	55.0

* Letters in italics refer to source of the culture as shown in footnote, table I.

gave results detectable from the 15-minute plates only in a slightly deeper color. Mounts of spores from the irradiated plates of these two sets of tests, and also from their controls, were then used as inoculum for petri-dish cultures, which after sufficient growth were given three daily 15-minute treatments with ultraviolet transmitted through vitaglass. Some of the previously irradiated plates and

their controls, one each of which had been used for the inoculum, were retained for comparison at the conclusion of the new tests. A control for the whole test was made by inoculating plates with mounts from the subcultures of the original unirradiated culture.

At the conclusion of the three days, the newly irradiated plates (A), obtained from the inoculum previously given three 30-minute exposures, showed a thick zonate light ochraceous salmon (16) pionnotes with many more spores than the controls, and with a deeper pionnotes than the plates from which the inoculum was taken; but there were still less than 1 per cent macrospores. The newly irradiated plates (B), whose inoculum came from the cultures previously given a 15-minute exposure period, also showed more spores than the control and than their inoculum plates; but there was more aerial flesh pink mycelium and a little less exposed pionnotes than on the A plates. Here again the microspores were more than 99 per cent of the total spore content. The newly exposed plates (C), with inoculum taken from the former control cultures, looked like the B plates and had a similar spore content. All unirradiated controls showed appressed moist or aerial white mycelium with no visible pionnotes and with a spore composition made up almost entirely of microspores.

A repetition of the experiment using plates A, B, and C for inoculum served to produce a slightly thicker pionnotes in all irradiated plates but no macrospore increase. The second subculture of the original unirradiated culture showed a very slight increase in spore production but no real pionnotes. Evidently the increase in spore content after irradiation was a little more than that ordinarily obtained by making subcultures from spores instead of from mycelium, for in every case treatment increased the total spore production more than did simple subculture by spore inoculum from the control. However, the tests failed entirely to produce a cumulative effect in percentage of macrospores present.

Similar results were obtained with strains of *F. conglutinans* and with *F. bulbigenum* forma 1 (*F. lycopersici*) which had also been unresponsive to early irradiation tests. In other strains of *F. bulbigenum* forma 1, which had been producing a few macrospores in stock tubes, repeated irradiation resulted in an increase in the total

spore production and in the number of macrospores present, but no appreciable increase in percentage. Thus the response seemed consistent regardless of whether the fungus treated was in "abkultur" or "hochkultur."

Results of irradiation through various filters

A distance of 40 cm. from the arc was found most advantageous for spore production. If this distance was shortened much, the factor of heat from the lamp entered in, and if it was lengthened much, the loss of the shorter rays became a factor. Although the distance from the arc and the length of exposure most favorable seemed to be about the same for most species tried, it was found that maximum production of spores was not induced in every strain when a vitaglass filter was used. This is brought out in figures 5 and 6 and in table III, which gives wave lengths transmitted by each filter and by the lamp itself. Figure 3 gives the spectrograms of the quartz mercury arc direct and through the various filters.

In the experiments with the filters, all plates were exposed for 15 minutes on each of three successive days at 40 cm. from the arc, but with the filter inserted in the lamp between the arc and the culture. During exposure the petri-dish cover was removed. Direct exposure was made without any filter in the lamp. Lids were removed from the controls also, for 15 minutes each day during exposure of the other plates. When not under the lamp, all plates were kept covered in diffuse light in the laboratory.

Although for most species tried, the vitaglass, transmitting wave lengths as short as 2650 A.u., gave maximum or very good results in spore production, maximum results with *F. vasinfectum* var. *zonatum* were always obtained by using Corex filter 980 A. (fig. 2 D), whose lower limit of transmission is 2300 A.u. Use of this filter gave as much as two or three times as high a percentage of normal macrospores as did the use of the vitaglass filter, although the latter filter induced three to eight times as high a percentage of macrospores as was present in the unirradiated plates (fig. 2 E). Use of Corex filter 986 A gave slightly better results with *F. vasinfectum* var. *zonatum* than did the vitaglass, and, although some normal spores as well as injured spores (fig. 2 F) occurred when the cultures were irradiated

TABLE III

EFFECT ON MACROSPORE PRODUCTION OF EXPOSING (AT 40 CM. FROM ARC FOR 15 MINUTES ON EACH OF THREE SUCCESSIVE DAYS)
FUSARIUM SPECIES TO ULTRAVIOLET RAYS EMITTED BY QUARTZ MERCURY ARC THROUGH VARIOUS FILTERS

SPECIES OF FUSARIUM	CONTROL (DIFFUSE DAYLIGHT)	NOVIOLE C 4850-6060 A.U.	G 586 AW 3334-3690 A.U.	FILTER NO. I 3020-6090 A.U.	VITAGLASS 2650-6090 A.U.	COREX 986 A 2535-4340 A.U.	COREX 980 A 2300-6090 A.U.	DIRECT. 2300-6090 A.U.
<i>F. decemcellulare</i> Brick. (section <i>Spicarioides</i>)	Macro. rare (8 mm.)†	Macro. rare (9)	Macro. rare (8)	Macro. less than ½% (8)	Macro. ½% * (8)	Macro. less than ½% * (6.5)	Less than ½% * (5.5)	No macro.; few micro. (4-under agar surface) 76.7% * (5)
<i>F. nivale</i> (Fr.) Ces. (<i>D-L</i>) (s. <i>Arachnites</i>)	59%§ (10)	61.3% (10)	63.5% (10)	61.8% (10)	75.2% * (10)	73.1% * (10)	66% * (10)	16% (7.5)
<i>F. sporotrichioides</i> Sh. (<i>B</i>) (s. <i>Sporotrichiella</i>)	10.9% (15.5)	10.5% (17)	9.6% (16.5)	11.8% (16)	51.7% * (16)	53.5% * (13.5)	34.3% * (12.5)	39.5% (?) (3-on surface 12-beneath)
<i>F. semitectum</i> B. & Rav. (<i>R</i>) (s. <i>Arthrosporiella</i>)	18.4% (15)	18.9% (16)	18.1% (15)	16.8% * (15)	18.8% * (15)	20% * (12)	25.3% * (13)	Macro. & micro. rare (2)
<i>F. scirpi</i> L. & F. var. <i>acuminatum</i> (E. & E.) Wr. (<i>R</i>) (s. <i>Gibbosum</i>)	51.9% (17.5)	55% (22.5)	64.6% (17)	62.2% (18.5)	85.7% * (17)	71.1% * (12)	71.9% * (12)	No spores
<i>F. sambucinum</i> Fuckel (<i>B</i>) (s. <i>Discolor</i>)	No spores (8)	No spores (7)	No spores (8)	Spores rare, mostly macro. * (7)	95% (7)	No spores (4)	No spores (5)	No spores (1)

* Total sporulation (macrospores and microspores) greater than controls.

† Letters in italics refer to source of culture as given in footnote, table I.

‡ Figures in parentheses express radial increase in mm. during 3 days following first exposure.

§ All percentages express percentage of macrospores present.

TABLE III—Continued

<i>F. neoceras</i> Wr. & Rg. (R) (s. <i>Lisciola</i>)	1.9% (14.5)	2.5% (14)	2.5% (15)	6.3% (14.5)	22.7% (14)	8.6% (14)	4.4% (14)	Macro. & micro. rare (11)
<i>F. orthoceras</i> App. & Wr. (W) (s. <i>Elegans</i> s.s. <i>Ortho-</i> <i>cera</i>)	No macro.; very few microspores (14)	Like control (14)	Like control (14)	No macro.; thin pion- notes * (15)	Less than 1%; * thin pion- notes (14)	Rare; * thin pion- notes (12)	Like 986 A * (8.5)	All spores rare (6.5)
<i>F. conglutinans</i> Wr. (W ₁₅) (<i>Elegans-Orthocera</i>)	No macro. (10)	No macro. (10)	Macro. rare (10)	Macro. rare (10)	Less than 1% * (10)	Rare * (9)	No macro. * (9)	No macro.; very few micro. (5)
<i>F. bulbigenum</i> Cke. & Mass. (W) (<i>Elegans-Constrictum</i>)	3.3% (14.3)	1.7% (15)	3.3% (15)	2.7% (15)	8.1% (13)	17.2% (12)	11.4% (11)	12.8% (6-mostly beneath agar)
<i>F. vasinfectum</i> Atk. var. zonatum (Sh) Wr. (W) (<i>Elegans-Oxyposium</i>)	5.6% (13.3)	5.1% (13.5)	5.6% (13.3)	10.9% (14)	17.5% (14.4)	19.2% (12)	37.7% (13)	17.6% (8.2-mostly under agar)
<i>F. javanicum</i> Koord. (R) (s. <i>Martella</i>)	3.4% (16)	4.2% (15.5)	5.6% (17)	6% (15.5)	10.2% (14)	9.2% (10)	8.6% (8)	Spores rare; many shat- tered (2 above agar 5 mm. total)
<i>F. javanicum</i> Koord. var. theobromae (App. & Strk.) Wr. (R) (s. <i>Martella</i>)	Macro. rare (17)	Macro. rare (16)	Macro. rare (16)	Macro. rare (17)	Less than 1% * (12)	Less than 1% * (8)	More than vital, but not 1% * (9)	Like 986 A but spores abnormal (3)
(<i>F. coeruleum</i> (Lib) Sacc. (S) (s. <i>Martella</i>)	No spores (6)	No spores (5.5)	No spores (6)	Few spores 95.6% (5)	Many spores 99.4% (5)	Fewer spores than vital. * 91.9% (3)	Like 986 A 85.6% (3.5)	No spores; mycelium empty or granular (1)

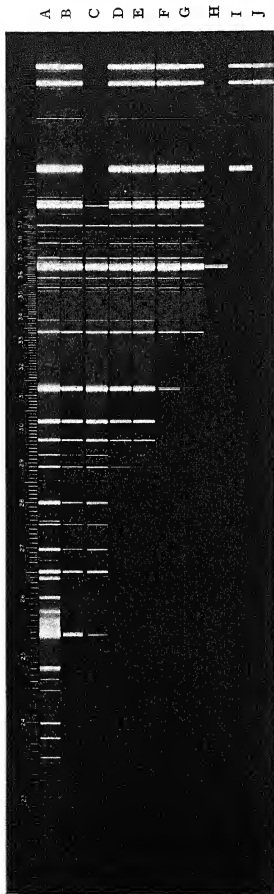


FIG. 3.—Spectrograms of quartz mercury arc direct and through various filters: A, direct arc (2230-6690 Å.); B, Corning Corex A (980 Å), (2300-6690 Å.); C, Correx red-purple (586 Å), 2535-4340 Å.; D, vitaglass (2650-6690 Å.); E, petri dish (2800-6690 Å.); F, glass filter no. 1 (3020-6690 Å.); G, glass filter no. 6 (3120-6690 Å.); H, Corning (G 586 Å), (3334-3690 Å.); I, Noviol A (4340-6690 Å.); J, Noviol C (4880-6690 Å.). Some faint lines showing lowest limits of transmission are lost in reproduction.

directly, usually the percentage was less than with any of the three filters transmitting as low as 2650 A.u.

The peak of macrospore production for *F. sporotrichioides* occurred consistently with the use of Corex filter 986 A (fig. 4 A), with which the shortest wave length transmitted was 2535 A.u. Although there was little difference between the results with this filter and those with vitaglass, the small advantage in number of macrospores produced was always on the side of the Corex 986 A (table III). Fewer sporotrichial, pyriform conidia (19, p. 183) occurred with 986 A than with vitaglass. When the fungus was exposed to the still shorter wave lengths transmitted by Corex filter 980 A, there was a decided drop in macrospore production but also in the sporotrichial conidia, the microspores present being ovate rather than pyriform. With filters transmitting no shorter waves than 3020 A.u. there was no noticeable change from the control plates, all of which produced few macrospores and a high percentage of pyriform conidia (fig. 4 B). *F. bulbigenum* Cke. and Mass. ran a much more evident peak under Corex filter 986 A than did *F. sporotrichioides*, for in every experiment the spore production here was much better and the macrospores produced (fig. 4 C) were of better shape than under vitaglass (fig. 4 D) and Corex 980 A. Sporulation on the control plates was almost entirely microspore (fig. 4 E).

In every instance, however, the fungi which developed maximum macrospore production under a filter transmitting shorter waves than 2650 A.u. gave increased sporulation with vitaglass also. Therefore this filter seems to be the most advantageous one to use for this purpose. The tests with *F. semitectum* B. & Rav., which were summarized for table III, show no increase in macrospore percentage under vitaglass, but in most individual experiments there was some increase, as is shown by the average in table I.

In the tests with *F. bulbigenum* another result is shown in the percentages cited in both table III and figure 5 for exposure directly to the arc without filters. It will be noted that the average percentage for direct exposure is slightly greater than that for Corex 980 A, making an irregularity in the curve. This irregularity was consistent for all individual experiments with *F. bulbigenum*, as well as for the averages, and was no doubt due to the lethal effects of

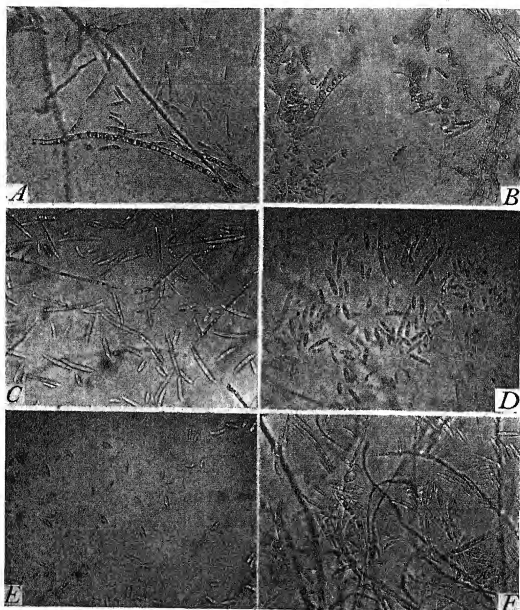


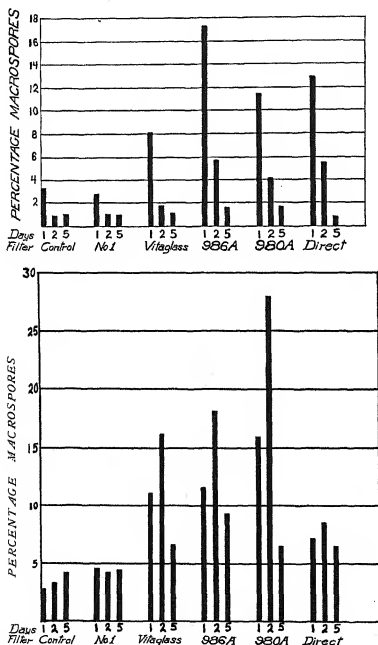
FIG. 4.—Effects of ultraviolet irradiation on cultures of *F. bulbigenum* and *F. sporotrichioides* placed 40 cm. from quartz mercury arc: A, increase in macrospores and decrease in sporotrichiate conidia in culture of *F. sporotrichioides* given three daily 15-minute exposures to Corex red-purple filter 986 A; B, control of *F. sporotrichioides* showing scarcity of macrospores and predominance of sporotrichiate conidia; C, abundance of macrospores of *F. bulbigenum* 24 hours after third daily 15-minute exposure through Corex red-purple filter 986 A; D, spores of *F. bulbigenum* present after three daily 15-minute exposures through vitaglass (2650–6690 A.u.); E, control of *F. bulbigenum* showing absence of macrospores; F, increase in microspore content of *F. bulbigenum* 48 hours after third daily 15-minute exposure through Corex red-purple filter 986 A (2535–4340 A.u.).

the shorter waves transmitted. In all plates exposed directly to the arc, this fungus showed a high percentage of spores whose contents were broken up into globules or which were apparently empty of protoplasm. Mycelium also exhibited a similar condition, but spores seemed to break up more readily and many spores were disrupted or badly shattered. Sometimes it was possible to recognize, for counting purposes, a macrospore which was disrupted or shattered, but shattered microspores could not be so easily detected. Often great masses of loose material or small clumps of floating glycogen and fat particles were suspected of coming from shattered spores, but could not, of course, be interpreted for counting. This decrease in detectable microspores could easily account for the upward trend of macrospore percentage when the plates were exposed directly to the arc.

Such a result occurred also with *F. nivale*, and with *F. semitectum*, which really had its maximum production of macrospores under Corex 980 A but which would seem from the average percentages shown in table III to be most favored by direct exposure. In reality *F. semitectum* was even more subject to destruction by the direct rays than was *F. bulbigenum*. The colony was much stunted and the spores badly shattered, again making a representative count impossible. Under Corex 980 A, on the other hand, the spores were normal.

All of the *Fusarium* species tried were injured somewhat by direct exposure to the arc: decided colony stunting, as measured by radial growth (figs. 7, 9, 10, 13); plasmolysis of protoplasts in mycelium and spores (fig. 2 *F.*); empty and abnormally shaped cells were noted. In some cells the protoplasm was present as a single comparatively uniform mass; in others numerous small refractive globules appeared. Microchemical reactions indicated the presence of glycogen and fat globules, some of which stained with Sudan III, others of which were partially saponified by sodium hydroxide. However, the severity of injury varied greatly with the species. *F. argillaceum* was extremely susceptible to injury by the shorter waves, those from the direct arc often causing the mycelium to go over almost entirely into production of chlamydospores, and stunt-

ing occurring even when the shortest waves were screened out by vitaglass. In some species there was little spore shattering under full transmission by the lamp, but spore production was checked so



FIGS. 5-6.—Fig. 5 (above), percentage of macrospores of *F. bulbigenum* present at end of one, two, and five days following irradiation with ultraviolet transmitted directly from quartz mercury arc and through various filters. Fig. 6 (below), percentage of macrospores of *F. vasinfectum* var. *sonatum* present at end of one, two, and five days following irradiation with ultraviolet transmitted directly from quartz mercury arc and through various filters.

that the actual number of spores present was lessened, and sometimes no spores occurred (as in *F. coeruleum*) or they were very rare (as in *F. scirpi* var. *acuminatum*). In other species, as in *F. javanicum* and *F. javanicum* Koord. var. *theobromae* (App. & Strk.) Wr., spores were produced between irradiations but were so shattered and abnormal after direct irradiation as to make representative spore count impossible. In no other case was injury as marked under vitaglass as was usually true in the case of *F. argillaceum* (fig. 1 F), although in some forms decrease in growth rate did occur with this filter, as is shown by the figures in table III expressing radial increase in millimeters during the three days following first exposures and by figure 8. Although decrease in growth rate sometimes occurred with this filter and often occurred with the two filters transmitting shorter waves (figs. 9, 10), only in rare cases was there any injury to spores and mycelium such as is described here for direct exposure.

In a number of species the total sporulation was somewhat augmented when irradiations were made through filter no. 1, which transmitted to 3020 A.u., and in some the percentage of macrospores was greater. The macrospore percentage of *F. neoceras* was tripled with filter no. 1 and that of *F. vasinfectum* var. *zonatum* sometimes doubled, but for the most part slight results were obtained with filters transmitting no lower than 3020 A.u. Increasing the length of exposure with this filter gave greater macrospore production with *Fusarium* spp., thus corroborating the findings of RAMSEY and BAILEY (14) for *F. cepae* and *Macrosporium*. The total sporulation produced under the three filters transmitting lower than 3020 A.u. was almost invariably greater than on unirradiated plates. Experiments with the Corex D filter in the Stewart health-lamp (wave lengths transmitted given under "Methods") gave essentially the same results as vitaglass. The following percentages of macrospores were obtained for the species named, in irradiated and non-irradiated plates respectively: *F. sporotrichioides*, given three daily 15-minute exposures, — macrospores 56.3%, control 36.5%; *F. semitectum*, exposed, — macrospores 6.5%, control 3.5%; *F. moniliforme* Sheld. var. *subglutinans* Wr. & Rg., exposed, — macrospores 7.2%, controls 4.6%; *F. orthoceras*, exposed, macrospores 1.2%, control (no macrospores); *F. vasinfectum* var. *zonatum*, exposed, — macro-

spores 46.7%, control 5%; and *F. argillaceum*, exposed, — macrospores 23%, control 22%.

In some species, as in *F. culmorum* previously mentioned, the results of irradiation were evident a week after irradiation, but in many species the effect was lost soon after the experiments were concluded. The germination of macrospores resulting in the production of many microconidia accounts for most of this change. In *F. bulbigenum* the drop in macrospore percentage for this reason became pronounced on the second day after irradiation ceased (figs. 4 F, 5), and by the fifth day all plates were essentially reduced to the same macrospore percentage as in the control plates. Consequently it was imperative for accurate comparison that all spore counts be made as near as possible to the end of the 24-hour period following irradiation.

In some of the other species the maximum production of macrospores was evident on the second day following irradiation, as in the case of *F. vasinfectum* var. *zonatum* (fig. 6). On the third day the percentage began to drop, and by the fifth day the percentages were nearing those of the controls. Usually the rise in macrospore count on the directly irradiated plates of *F. vasinfectum* var. *zonatum* was especially noticeable, although in the experiments averaged in figure 6 the increase is not striking.

Effect of ultraviolet on *Fusarium* species with proven perfect stage

Some tests were made with those species of *Fusarium* with proven perfect stages, to see whether irradiation with ultraviolet would induce perithecial formation. The results of these experiments are summarized in table IV and need little discussion. The 4-day old cultures were treated as in the previous tests with three 15-minute exposures at 40 cm. from the mercury arc, the rays passing through the vitaglass filter. Essentially the same results were obtained whether the filter was in the lamp or used as a petri-dish cover. A duplicate set of tests was run at the same time, however, in which the cultures were exposed to the direct rays from the mercury arc for 4 seconds at a distance of 21 cm. from the arc. This latter set was run since STEVENS (20) obtained many perithecia of *Glomerella*

cingulata (Stoneman) Sp. & Schrk. by exposing them to direct rays for 4 seconds at 21 cm.

While the results on conidia production were essentially as one would expect from previous experiments, no perithecial development was evident at the conclusion of the experiments. A few tests with 1 second and 5 seconds direct and with 15-minute exposures under the various filters were also negative. STEVENS found the rudiments of perithecia of *Glomerella cingulata* 15 hours after irradiation and obtained mature ascospores in 4 days. In the case of *Colletotrichum lagenarium*, although the plexi began to develop 24 hours or less after irradiation and the perithecia 1-10 days later, he found that sometimes the perithecia did not appear for 23-30 days. Therefore the species of *Fusarium* here tested were held for several weeks to two months. Only in the case of a subculture of REINKING's strain 129 (33, p. 168) of *F. javanicum* Koord. var. *theobromae* (App. & Strk.) Wr. did perithecia develop at all, and then only in one experiment. In the work with two strains of this fungus the mature perithecia of the perfect stage, *Hypomyces ipomoeae* (Hals.) Wr., were found seven weeks after irradiation on two plates of one strain which had been thrice irradiated for 15 minutes; and vestigial perithecia were found in one of the second-strain cultures which had been irradiated directly for 4 seconds on each of three successive days. Other plates similarly treated bore no perithecia, and those found on the plates mentioned occurred only on the glass sides of the petri dishes at the edge of the colony, in the new growth since the irradiation; therefore although controls were also negative there was no real evidence that ultraviolet rays were responsible for the occurrence of the few scattered perithecia which appeared in one test.

In another strain of *Hypomyces ipomoeae*, which was producing perithecia in artificial culture, similar tests brought no increase in perithecial production over the control plates which also produced mature perithecia. It may be worthy of note that growing WINELAND's two strains (27) of *F. moniliforme* together under ultraviolet radiation did nothing to restore their erratic tendency to produce the perfect stage, *Gibberella moniliformis* (Sh.) Wineland. These subcultures were not, however, producing the "blue-black globoid

TABLE IV
SUMMARY OF EXPERIMENTS SHOWING EFFECTS OF ULTRAVIOLET ON SPECIES OF FUSARIUM WITH KNOWN PERFECT STAGE

SPECIES OF FUSARIUM	PERFECT STAGE	RESULTS OF IRRADIATION		CONTROL
		4 SEC. DIRECT, 21 CM. FROM ARC	15 MIN. THROUGH VITAGLASS, 40 CM. FROM ARC	
<i>F. decemcellulare</i> Brick. (R)* (s. <i>Spicarioides</i>)	<i>Calonectria rigidiuscula</i> (B. & B.) Sacc.	No colony stunting; no perithecia, no macrospores; fewer microspores than control	No colony stunting; no perithecia, no macrospores; more micro. than control	No perithecia; only O-septate spores
<i>F. nivale</i> (Fr.) Ces. (D-L) (s. <i>Arachnites</i>)	<i>Calonectria graminicola</i> (B. & B.) Wr.	No colony stunting; no perithecia; 70.5% macro; deeper color than control	No colony stunting; no perithecia; 84.8% macro; deeper color than control	Few spores; no perithecia 35.5% to 60.2% macrospores
<i>F. equiseti</i> (Cda) Sacc. var. <i>bulbatum</i> (Sherb.) Wr. (R) (s. <i>Gibbosum</i>)	<i>Gibberella intricans</i> Wr.	No stunting; no perithecia even when held several weeks; no macrospores	Same as 4" direct	No perithecia; no macrospores
<i>F. sambucinum</i> Fuckel (1 strain from S and one from potato. Results identical)	<i>Gibberella pulcaris</i> (Fr.) Sacc.	Much stunting; no perithecia even when held 2 months; spores none to rare macrospores	Much stunting; no perithecia in 2 months; spores few to moderately plentiful; 100% macro.	No perithecia in 2 months; no spores
<i>F. graminearum</i> Schwabe (2 strains W-L) (s. Discolor <i>Saubinetii</i>)	<i>Gibberella saubinetii</i> (Mont.) Sacc.	Slight stunting; no perithecia in several weeks; 100% macrospores	No stunting; otherwise as for direct	No perithecia; 100% macrospores
<i>F. lateritium</i> Nees. var. <i>mori</i> Desm. (W) (Syn. <i>F. urticarum</i> (Cda) Sacc.) (s. <i>Lateritium</i>)	<i>Gibberella baccata</i> (Wallr.) var. <i>moricola</i> (Ntrs) Wr.	No stunting; total sporulation greater than control; 1.3% macrospores	2% macrospores; otherwise as direct; no perithecia in either	Less than 1% macrospores; no perithecia

* Letters in italics refer to source of culture as given in footnote, table I.

TABLE IV—Continued

F. moniliforme Sheldon (Wineland's Y 29) (J)	Gibberella moniliformis (Sh.) Winel.	No stunting; no perithecia in 2 months; more spores than control but macrospores rare	No stunting; no perithecia in 2 months; more spores than direct; 1% macrospores	No perithecia in 2 months; macrospores rare
Wineland's Y 15 (J)	Gibberella moniliformis (Sh.) Winel.	Slight stunting; no perithecia in 2 months; occasional macrospore; fewer than with vitaglass	Slight stunting; no perithecia in 2 months; less than 1% macrospores	No perithecia in 2 months; macrospores rare
Y 29 & Y 15 on same plate		No perithecia in 2 months	As for direct	No perithecia
F. moniliforme Sheld. var. minus Wr. & Rg. (K) (s. Liseola)	Gibberella fujikuroi (Saw.) Wr.	Slight stunting; no perithecia in 2 months; more spores than control; less than 1% macrospores	As for direct except thicker pionnotes and 2.1% macrospores	No perithecia in 2 months; macrospores rare
F. javanicum Koord. var. theobromae (App. & Strk.) Wr. (R)	Hypomyces ipomoeae (Hals.) Wr.	<i>At conclusion of irradiation period</i> No stunting; no macrospores; no perithecia; fewer micro. than control	Slight stunting; few macrospores; no perithecia	No macrospores; no perithecia
		<i>7 weeks after irradiation period</i> A few macrospores; no perithecia	A few macrospores; perithecia at edge of colony on side of petri dish only	A few macrospores; no perithecia
F. javanicum Koord. var. theobromae (App. & Strk.) Wr. (R) 2nd strain from (K) labeled	"Hypomyces ipomoeae" but bearing no perithecia on receipt	<i>At conclusion of irradiation period</i> Slight stunting; no macrospores; no perithecia	Slight stunting; macrospores less than 1%; no perithecia	No macrospores; no perithecia

TABLE IV—Continued

SPECIES OF FUSARIUM	PERFECT STAGE	RESULTS OF IRRADIATION		CONTROL
		4 SEC. DIRECT, 21 CM. FROM ARC	15 MIN. THROUGH VITAGLASS, 40 CM. FROM ARC	
		<i>7 weeks after irradiation period</i>		
		A few macrospores and a few vestigial perithecia on edge of petri dish only	A few macrospores; no perithecia	
<i>F. javanicum</i> Koord. var. <i>radicicola</i> Wr. (R)	<i>Hypomyces ipomoeae</i> (Hals.) Wr. f. 1 Wr.	No stunting; 2.1% macrospores; no perithecia even after 2 months	No stunting; 5.3% macrospores; no perithecia in 2 months	Less than 1% macrospores; fewer microspores than on exposed plates; no perithecia
<i>F. solani</i> (Mart. pr. p.) App. & Wr. var. <i>eumartii</i> (Carp.) Wr. (S)	<i>Hypomyces haematococcus</i> (B. & Br.) Wr.	Some stunting; microspores only; no perithecia in 2 months	Slight stunting; microspores only; no perithecia in 2 months	Microspores only; no perithecia in 2 months
<i>F. solani</i> App. & Wr. var. <i>minus</i> Wr. (R)	<i>Hypomyces haematococcus</i> var. <i>breviconus</i> Wr.	No stunting; no macrospores; no perithecia in 2 months	No stunting; less than 1% macrospores; no perithecia in 2 months	Microspores only; no perithecia in 2 months
<i>F. solani</i> App. & Wr. var. <i>striatum</i> (Sherb.) Wr. (s. Martiella)	<i>Hypomyces haematococcus</i> var. <i>cancrini</i> (Rutg.) Wr.	No stunting; only microspores; no perithecia in 2 months	As for direct	Microspores only; no perithecia in 2 months
<i>F. argillaceum</i> (Fr.) Sacc. (W)	<i>Hypomyces solani</i> Rke. & Berth.	Colony very stunted; macrospores none to rare; no perithecia in 3 months; many chlamydospores	Colony stunted; spores few but mostly macrospores (60-70%); no perithecia in 3 months; many chlamydospores	Spores usually more plentiful in control but 20-40% macrospores; no perithecia in 3 months; chlamydospores rare

bodies" which she found present when they were in good condition for pairing and resultant production of perithecia.

Decrease in growth rate and sporulation

Although there was in practically every instance much colony stunting owing to decrease in growth rate and lethal effects of the shorter rays when the cultures received the full emanations from the lamp, the amount of stunting which occurred with the several filters was variable, as is shown in tables III and IV. The stunting following direct exposure was always accompanied by lethal effects, such as plasmolysis of cells in the mycelium or spore destruction; but only with certain species were such conditions evident upon microscopic examinations of the cultures exposed to rays passing through the filters. The decrease in growth rate, as measured by the radial increase in millimeters during the three days following the first exposure, was usually very slight or lacking when vitaglass was used, and usually greater when the Corex filters 986 A and 980 A were used; but this varied for the species irradiated. It will be noted from table III that *F. nivale* and *F. neoceras* usually showed no stunting except upon direct exposure, but occasionally in the same experiment one plate would show stunting under a given filter and the others not (fig. 12). This was not correlated with variations in percentage of macrospores. Thus not only was there variation among the different organisms in this matter but sometimes the same strain reacted differently. *F. decemcellulare* sometimes showed slightly decreased growth rate under vitaglass, usually not; *F. javanicum* (fig. 11) and *F. javanicum* var. *theobromae* (fig. 7) usually showed some stunting when this filter was used, but sometimes not. *F. vasinfec-tum* var. *zonatum* usually showed no stunting with vitaglass and often none with the two filters transmitting shorter wave lengths; but sometimes there was slight stunting of the colonies under these last filters (fig. 13). There was always stunting with direct exposure, and usually the radial increase after direct irradiation was begun was largely beneath the surface of the agar, the agar screening out the detrimental shorter waves. This was also true of a great many of the other species. For example, the radial increase during the three days following the first direct exposure of *F. semitectum* was 12 mm.

beneath the surface of the agar and only 3 mm. on the surface, while that of the controls was 15 mm. on the surface. In *F. coeruleum* there was almost no growth above the surface on direct plates (fig. 9; table III). In every experiment normal growth was resumed from sub-agar mycelium or aerial mycelium a day or two after cessation of irradiation.

Although decrease in growth rate and macrospore production were often associated, there were so many exceptions that there seemed to be no definite correlation between the production of macrospores after irradiation and inhibition of growth. Therefore, the results of irradiating many species of *Fusarium* would seem to corroborate the findings of RAMSEY and BAILEY (14) for *Macrosporium* and for *F. cepae*, that increased spore production subsequent to irradiation with ultraviolet is not a direct result of inhibition of growth rate. Surely inhibition of growth rate is not alone responsible, for decreasing the rate of growth by exposure to higher or lower temperatures or by using less favorable media did not produce the same results. Through personal correspondence with Miss STRONG and G. H. COONS, the writer learned that in no case did abundant sporulation accompany the inhibition in growth rate of *Fusarium* species owing to the toxic effect of dyes (2).

HUTCHINSON (6), in his work with the response of *Colletotrichum phomoides* to ultraviolet, found similar lack of correlation between the growth rate and sporulation. With full mercury arc irradiation he always got a retardation of growth. However, this was accompanied by increased sporulation only under exposures of 15 seconds to 2 minutes, whereas sporulation decreased when exposures were for more than 2 minutes. He considered this as an indication that sporulation is an inverse expression of growth rate only within certain limits, and that there is an optimum amount of irradiation causing sporulation. He states that certain monochromatic bands retarded growth and stimulated production of acervuli while with other wave lengths there was initial retardation of growth followed by stimulation and also hastening of sporulation. Certain waves constantly stimulated growth and had no apparent effect upon the time of development of spores; but on the other hand wave lengths

4078 A.u. and 5819 A.u., which produced the most marked stimulation of growth, also caused early development of acervuli. Therefore he concluded that retardation and extreme stimulation of growth are accompanied by early sporulation, while irradiations causing intermediate stimulation of growth have no apparent effect in the development of acervuli.

In the present work with *Fusarium*, some species, such as *F. moniliforme*, *F. bulbigenum* forma 1, etc., which showed a decrease in the size of the colony when irradiated, showed no increase in macrospore production. On the other hand, the radial increases given in table III show that macrospore production was greatly increased when *F. nivale* (fig. 1 C), *F. sporotrichioides* (fig. 14), *F. scirpi* var. *acuminatum*, *F. neoceras* (fig. 12), and *F. vasinfectum* var. *zonatum* (fig. 13) were subjected to ultraviolet radiation through vitaglass, even when there was no decrease in the rate of growth during this treatment.

Irrespective of whether inhibition of growth of *F. vasinfectum* var. *zonatum* took place under filters 980 A and 986 A, an increased percentage of normal macrospores always occurred. In contrast, *F. javanicum* var. *theobromae* (fig. 7), which showed much stunting under these two filters, showed very little spore increase; in fact, the vitaglass plates, which showed decrease in growth rate but no microscopic evidence of spore destruction, often showed less total sporulation than the control plates. A culture of *F. sambucinum*, treated for 4 seconds direct on three successive days, grew only 3 mm. during those three days; whereas the controls averaged 13-mm. growth but neither set of plates showed any spores; yet duplicate cultures given three 15-minute exposures to rays no shorter than 2650 A.u. likewise grew only 3 mm. and showed considerable spore production. *F. equiseti* var. *bullatum* sometimes exhibited decreased growth after 15-minute irradiation periods, sometimes not; but in neither case did irradiation increase macrospore production. And in the one experiment where there was considerable decrease in colony size, the control plates averaging 48 mm. diameter at the conclusion of the experiment and the plates that received 15-minute irradiation with waves of 2650 A.u. and above averaging 28 mm.

diameter, the control plates showed a greater total spore production than the exposed ones, and rare macrospores as against no macrospores on the treated plates.

These data and the fact that the acme of spore production, except in a few species, occurs with the vitaglass filter which gives no detectable decrease or, at least, the smallest decrease in growth rate (as measured by radial increase of the colony and by observation of amount of aerial mycelium present), would seem to indicate that the increase in macrospores was the result of stimulation effected by ultraviolet rays rather than an indirect result of the inhibition in growth. Further apparent evidence for this conclusion lies in the fact that if a fungus were one responsive to treatment with ultraviolet, old petri-dish cultures of it, which had completely filled the plate and so were arrested in growth, could be made by irradiation to produce spores even on the old mycelium.

What changes take place in the mycelium subsequent to irradiation and immediately preceding sporulation have not yet been worked out. Tests for change in pH of mycelium or of medium as a result of irradiation were negative. No tests were made for the presence of hydrogen peroxide, although it is known that some workers consider the lethal action of ultraviolet to be an indirect one due to development of hydrogen peroxide in the nutrient substrate exposed to the rays. COONS (1) found that he could use a dilute solution of hydrogen peroxide to replace the light stimulus in the production of fruit bodies of *Plenodomus fuscomaculans*. He states that the action of light was modified and controlled by the condition of the mycelium, which is a resultant of all the acting environmental factors. Macroscopically undetectable differences in mycelium condition may also account for the variation in reaction of *Fusarium* species to ultraviolet, and it is thought that microchemical studies of the mycelium previous to and subsequent to irradiation of responsive species may throw considerable light on this question.

No work was done with intensity of the ultraviolet rays involved, since the object of this study was primarily to determine the usefulness of ultraviolet radiation in taxonomic studies of *Fusarium*. RAMSEY and BAILEY had found in the work on *F. cepae* and *Macrosporium* that, while intensity was an important factor, it apparently

is not primarily responsible for the variation between the different filters in amount of increase in sporulation. SWANN and DEL ROSARIO (26), working with *Euglena*, found that the predominance of the lethal effect of one monochromatic ultraviolet line over that of another given line "is not explicable entirely as a matter of absorption, but involves, in addition, other characteristics of the wave lengths concerned."

Discussion

While the chief value of ultraviolet irradiation of *Fusarium* possibly lies in inducing the production of macrospores of certain species when the macroconidia are lacking in the strains under observation, there is also some promise that ultraviolet radiation may be useful for further verification of systematic identification of some species. The consistency of response to ultraviolet of certain forms which characteristically produce macrospores or fail to produce macrospores upon irradiation seems of some diagnostic value. It is interesting to note in this respect that of the ten strains of *F. moniliforme* and three authentic varieties exposed to ultraviolet treatment, none gave noticeable increase in percentage of macrospores; while *F. neoceras* (33), which is the outstanding species morphologically in section Liseola, also stands definitely apart from the other forms of the same section in its response to ultraviolet by abundant macrospore production. *F. argillaceum* also, which stands alone as the only species in its section, is the only species tested which was greatly injured by the rays passing through vitaglass and which showed the striking tendency to go over into chlamydospore production as a result of irradiation. The contrast in response between vascular parasites and other species in section *Elegans* has already been discussed.

Variation in reaction to the various filters is scarcely sufficient to be of help in separating species, but the responsiveness or non-responsiveness to macrospore production and the variation in colors intensified by exposure to ultraviolet might easily be of some help in classification. In colored *Fusarium* species, such as *F. bulbigenum* (fig. 8) and *F. coeruleum* (fig. 9), the colors became increasingly intense with filters transmitting lower wave lengths. In the case of some species whose mycelium was white in culture tubes, colors

developed under irradiation by the shorter rays. The mycelium of *F. javanicum*, for instance, which was white under filters transmitting no lower than vitaglass (fig. 11), became pale violet grey to pale glaucous blue above a white to pearl grey background when irradiated through filter 986 A, deepened to deep glaucous grey under filter 980 A, and was dusky green blue (2) to green blue slate when irradiated direct (fig. 10). It therefore stood out in marked contrast to its variety, *F. javanicum* var. *theobromae*, which remained white even when irradiated directly (fig. 7).

It is felt that while the use of response to ultraviolet radiation as an aid in differentiation of *Fusarium* species is of less value than morphological and pathogenicity studies, it may add one more physiological test in instances of difficult diagnosis. The value of irradiation for inducing macrospore production in short time in many species is beyond question. It must be realized, however, that it cannot always be applied, for there are many cases in which it fails to induce macrospore production.

Summary

1. In most of the 59 species, varieties, and forms of *Fusarium* exposed to the ultraviolet rays transmitted by a Cooper-Hewitt quartz mercury-arc lamp through filters transmitting waves as low as 2650 but no lower than 2300 A.u., there was an increase in total sporulation (macrospores and microspores) if not in the percentage of macrospores present. This increase was usually evidenced by the production of a pionnotes or of sporodochia; or at least by greater intensity of mycelium, stroma, and spore mass colors. Colors not present in the control developed in the mycelium of some forms when exposed to the shorter rays of ultraviolet.

2. In some species there was a slight increase in spore production with filters transmitting no lower than 3020 A.u., but with filters transmitting no ultraviolet results were negative.

3. Some species, such as *F. neoceras*, *F. cepae*, *F. solani* var. *medium*, *F. sporotrichioides*, *F. vasinfectum* var. *zonatum* and forma 1 and 2, *F. bulbigenum*, and *F. redolens*, always gave very striking increase in macrospore percentage with three daily 15-minute treatments under vitaglass (transmitting to 2650 A.u.).

4. Some other species gave favorable although not such striking results in macrospore increase.

5. Many species, such as *F. conglutinans*, *F. bulbigenum* forma 1, *F. moniliiforme* and its varieties, *F. orthoceras* and its varieties, *F. oxysporum*, etc., showed very little or no macrospore increase after irradiation.

6. Except in the cases of *F. scirpi* var. *acuminatum*, *F. javanicum*, and *F. argillaceum*, the tendency to respond or not to respond to irradiation with macrospore production was consistent and seemed characteristic of the species or variety.

7. All of the species which responded to the treatment, except *F. oxysporum* var. *nicotianae*, were saprophytes or decay producers. The culture of *F. oxysporum* var. *nicotianae* was one which had not been tested for pathogenicity.

8. All of the known vascular parasites which were tested failed to show appreciable macrospore increase as a result of irradiation.

9. The most extensive spore production induced by irradiation occurred in species of sections Sporotrichiella, Gibbosum, Discolor subsection Saubinetii, Martiella, and Elegans subsection Oxy-sporum (excluding vascular parasite group).

10. Initiation of sporulation in *F. culmorum* was hastened. Irradiated plates showed as high as 83 per cent macrospores whereas controls showed no conidia, and even after the controls began to sporulate, treated plates showed a higher percentage of macrospores.

11. A strain of *F. coeruleum*, which had never produced conidia during its period of culture in this laboratory, and one of *F. sambucinum*, which was temporarily in "abkultur," were induced to produce macrospores after irradiation, although the controls were still sterile.

12. One strain of *Fusarium* (section Gibbosum), isolated from decayed onion bulbs, which had never produced spores in the laboratory, produced many spores (75 per cent macrospores) when irradiated. Subsequent irradiation failed further to increase this percentage.

13. There was little or no cumulative increase in macrospore percentage due to repeated irradiation of non-responsive species, although the quantity of spores present could be thus increased.

14. *F. vasinfectum* var. *zonatum* and *F. semitectum* gave maximum macrospore production under Corex filter 980 A transmitting to 2300 A.u. With *F. sporotrichioides* and *F. bulbigenum* the peak of macrospore production occurred under Corex 986 A transmitting between 2535 and 4340 A.u.

15. Most species tested gave maximum macrospore production under vitaglass. All responsive species gave good results under vitaglass.

16. A 15-minute irradiation period on each of three successive days was better than one longer period. A shorter period was usually not so effective. In some species increasing the length of period to one half-hour, or multiplying the number of 15-minute exposures was advantageous, in others injurious.

17. A distance of 40 cm. from the arc proved best for the purposes of these tests.

18. Exposure directly to the mercury arc, transmitting to 2230 A.u., resulted in decreased growth rate (as measured by radial increase), colony stunting, and injurious effects to mycelium and spores in all species. The extent of the injurious and lethal effects varied with the fungus. In some forms spore formation was inhibited, in others those produced were killed, or injured or deformed. Plasmolysis of protoplasm and shattering of spores occurred.

19. Most forms tried were injured slightly or not at all by the exposure to waves transmitted through the filters, though decrease in growth rate occurred with some species under some filters.

20. Increased macrospore production did not seem to be directly correlated with inhibition in growth rate.

21. A stimulation of macrospore production resulted upon irradiation of old mycelium in cultures of responsive species, although the mycelium already completely filled the petri dish and was checked in growth.

22. *F. argillaceum* usually produced many chlamydospores as a result of irradiation. Cultures exposed directly to the arc sometimes went over almost entirely to chlamydospore production.

23. In some species, as in *F. vasinfectum* var. *zonatum*, macrospore content was greatest 48 hours after irradiation ceased, and there was a drop in percentage subsequently owing to the production of micro-

spores on germination of the macrospores. In other species, as in *F. bulbigenum*, maximum macrospore percentage occurred 24 hours after the third exposure and a drop in percentage was noticeable the second day.

24. Neither three 15-minute exposures through vitaglass at 40 cm. from the arc nor three (or less) 4-second direct exposures at 21 cm. from the arc succeeded in inducing the production of perithecia of the 14 species with known perfect stages which were treated in these tests. Except for two plates in one test of *F. javanicum* var. *theobromae*, none of the cultures tested produced perithecia. In these two plates a few perithecia were present two months after irradiation in mycelium developed since irradiation.

The writer wishes to express thanks to Dr. G. B. RAMSEY for help and criticism during the course of the work and to Dr. G. K. K. LINK for encouragement and for criticism of the manuscript.

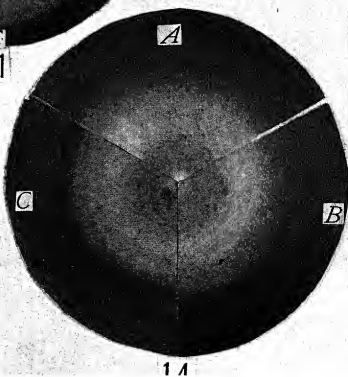
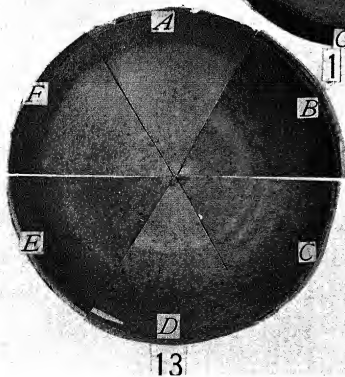
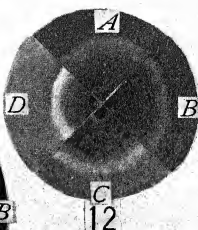
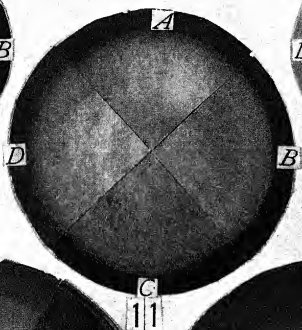
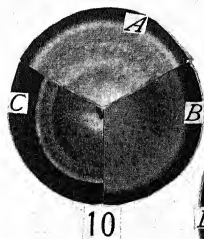
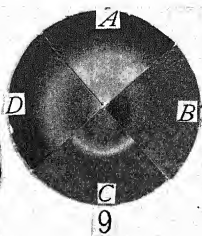
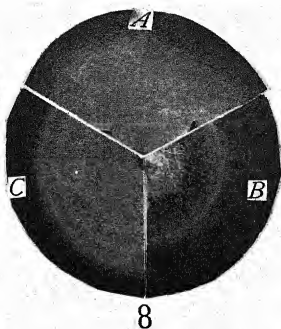
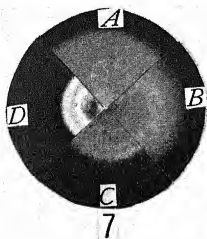
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EXPLANATION OF PLATE I

Segments of petri-dish cultures of *Fusarium* spp. showing comparative growth rates as affected by three daily 15-minute exposures to ultraviolet rays transmitted by quartz mercury arc through various filters (distance 40 cm.).

FIG. 7.—*F. javanicum* var. *theobromae*: A, control grown in diffuse light; B, exposed through vitaglass; C, exposed through Correx red-purple filter (986 Å); D, exposed direct.

FIG. 8.—*F. bulbigenum*: A, control; B, exposed through filter 986 Å; C, exposed through vitaglass.

FIG. 9.—*F. coeruleum*: A, control; B, exposed direct; C, exposed through Corning Correx filter (980 Å); D, exposed through vitaglass.

FIG. 10.—*F. javanicum*: A, exposed through filter 986 Å; B, exposed through filter 980 Å; C, exposed direct.

FIG. 11.—*F. javanicum*: A, control; B, exposed through filter No. 1; C, exposed through vitaglass; D, exposed through Noviol C.

FIG. 12.—*F. neoceras*: A, control; B, exposed through vitaglass (no stunting); C, exposed through filter 986 Å and showing no stunting; D, exposed through filter 986 Å but showing stunting. Both plates sporulated equally well.

FIG. 13.—*F. vasinfectum* var. *zonatum*: A, control; B, exposed direct; C, exposed through filter 980 Å; D, exposed through filter 986 Å; E, exposed through vitaglass; F, exposed through filter No. 1.

FIG. 14.—*F. sporotrichioides*: A, control; B, exposed through vitaglass; C, exposed through filter 986 Å.

GAMETOGENESIS IN VAUCHERIA

JOHN N. COUCH

(WITH THIRTY-FIVE FIGURES)

Introduction

A review of the literature on gametogenesis in *Vaucheria* indicates that there are two important problems connected with this process about which there is still considerable doubt. First, is fertilization effected in all species by means of motile sperms, or does the antheridium discharge directly into the oogonium? Second, what happens to the supernumerary nuclei in the oogonium? Do they migrate back into the thread as maintained by OLTMANNS (11) and HEIDINGER (6), or do they degenerate in the oogonium as claimed by DAVIS (4) and MUNDIE (9)?

VAUCHER (16) seems to have been the first who in any way understood the development and function of the sexual organs of *Vaucheria*. According to WALZ (17) the female sexual organ had been seen before VAUCHER, although it was not understood. VAUCHER discovered the male organ. Concerning fertilization he says (free translation):

At first the antheridium is straight and opaque as a consequence of the contained fertilizing matter; little by little it curves toward the oogonium in order to deposit the fertilizing element on the latter. When the fertilizing element is discharged, the antheridium is empty and twisted in a spiral.

NÄGELI (10) was the next to attempt to describe the sexual process in *Vaucheria*. His observations are interesting chiefly from a historical standpoint. NÄGELI considered the antheridia and oogonia as organs of fertilization, the process being similar to that in the Zygnemaceae (fig. 1).

PRINGSHEIM (12) described accurately for the first time the development of the sexual organs. Studying the same species as NÄGELI (*V. sessilis*), PRINGSHEIM saw the formation of the sperms, their discharge, and fertilization. So accurate were his observations on living material that even today one can add little to his account

of the development of the sperms and fertilization. Quoting from a free translation:

After the formation of the dividing wall in the antheridial branch ("Hornchen"), the colorless slime in its end gradually takes on a more distinct form, and one can easily recognize a great number of little rods in different places, close together and lying on top of one another, entirely colorless, which are still here and there surrounded by slime as though imbedded in it. The sharp observer will not miss the indistinct movement which some of these little rods show, and which gives a foreboding of their destiny.

PRINGSHEIM describes the discharge of the sperms:

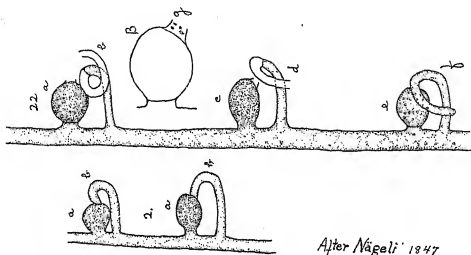
Then always immediately after the opening of the oogonial beak, in wonderful coincidence, the antheridium opens at the end and pours out its contents. Countless extremely small, rod-shaped bodies emerge, some isolated and others still in the slime in which they were imbedded. Those already isolated glide about in all directions with incredibly quick movements; those imbedded in the slime gradually become free and follow with equal speed. Soon the field of view is covered with the minute, moving rods. In considerable numbers, 20, 30, and more, they push into the nearby opening of the spore fruit, which they completely fill.

He further describes how these sperms in the beak process of the oogonium push against the surface of the egg, attempting to penetrate it: "The sperms strike violently, fall back, push forward again, fall back again, and so continue in uninterrupted succession their pushing forward and falling back." He did not observe the actual penetration of a sperm into the egg, but saw what he took to be a sperm just after it had entered the egg.

The observations of PRINGSHEIM on *Vaucheria sessilis* are of interest because they are the first record of the union of a sperm with an egg in plants. The following year (1856) PRINGSHEIM actually watched the sperm enter the egg in *Oedogonium* (JOHNSON 7).

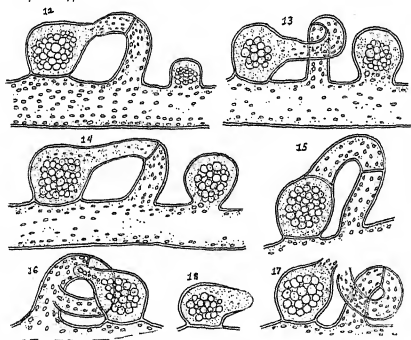
DE BARY (1) described the development of the oogonia and antheridia and fertilization in *Vaucheria aversa*. He too observed the movement of the sperms in the antheridia, their discharge, and the probable entrance of a sperm into an egg. In the same year (1856) DIPPEL (5) maintained that PRINGSHEIM was wrong and NÄGELI right in the development of *Vaucheria*. He claimed that the so-called "Hornchen" (antheridium) of PRINGSHEIM fused with the beak of the sporangium (oogonium); that the cross walls between these

two organs disappeared; and that part of the contents of the "Hornchen" passed over into the sporangium (oogonium). After this the "Hornchen" broke away from the oogonial beak, bending around



After Nägeli 1847

From Flora 1856 Part I Plate V
After Dippel



FIGS. 1, 2.—Fig. 1 (above), NÄGELI's figures of conjugation in *Vaucheria*. Fig. 2 (below), some of DIPPEL's figures of conjugation in *V. sessilis*.

and assuming such a position that one could hardly suspect their former connection. The "Hornchen," however, did not always become separated from the oogonium but sometimes remained at-

tached. DIPPEL's observations were made on *V. sessilis*, of which he presented a number of figures in support of his view. Figure 2 shows part of one of DIPPEL's plates, illustrating fertilization in *Vaucheria*.

The observations of PRINGSHEIM and DE BARY were confirmed by SCHENK's (13) study of *V. geminata*.

WALZ (17) described fertilization in *V. sericea* Lyngb. His work is in essential detail a confirmation of that of PRINGSHEIM, DE BARY, and SCHENK. He seems to have been the first actually to observe the penetration of the sperm into the egg in *Vaucheria*.

SOLMS-LAUBACH (15) described the development of the sexual organs and fertilization in *Vaucheria dichotoma*, and WORONIN (18, 19) did the same for *V. synandra* and *V. de baryana*, both writers corroborating, in a general way, the observations of PRINGSHEIM, DE BARY, and others. WORONIN (18) described the sperms of *V. synandra* as being somewhat elongated bodies, 5 to 6 μ in length, with two cilia arising from near the anterior end, one being directed forward and the other backward. According to WORONIN, the sperms contain one or two very small, shiny, colorless bodies, corresponding perhaps to the red pigment dots which were seen by DE BARY and WALZ in *V. aversa*.

The observations so far described were made almost entirely on living material. The new killing, fixing, and staining technique developed about this time enabled SCHMITZ (14) to demonstrate the nuclei in *Vaucheria* for the first time.

The second problem in *Vaucheria* concerns the fate of the supernumerary nuclei in the oogonium. SCHMITZ stated that the young oogonium contained many nuclei and that the young oospore contained a single large nucleus. He did not offer any explanation, however, as to the fate of the extra nuclei. Later he claimed (according to MOTTIER 8) that, in the plasmic mass given out upon the opening of the oogonium, small nuclear fragments were present which had probably become separated from the nuclei of the young oogonium.

BEHRENS (2) stated that the numerous nuclei in the oogonium fused to form one. He also noticed but did not understand the significance of the "Wanderplasm" which OLTMANNS later described.

OLTMANNS (11), using modern cytological technique, was the first to give a complete account of the development of the sexual organs,

including the nuclear behavior. He described in great detail the development of the sperms in *V. clavata* (*V. sessilis*), *V. fluitans*, and *V. aversa*, in both living and fixed material. According to OLT-MANNS, the young antheridium contains several large vacuoles bordered by a rather dense cytoplasm in which many nuclei are imbedded. These nuclei elongate and become spindle-shaped, one end extending out into the vacuole and the other end being imbedded in the peripheral cytoplasm. Each of these nuclei with a small amount of surrounding cytoplasm becomes a sperm. Cilia may sometimes be observed on the sperms. Shortly before the antheridium opens, the sperms may be seen in motion within it. When the antheridium opens, the sperms emerge with great rapidity. The peripheral plasma layer may remain partly within the antheridium, even after the sperms are discharged. The antheridia generally open between 2 and 4 o'clock in the morning. The most remarkable of OLT-MANNS' observations was in the development of the oogonia. The young oogonia contain a great number of nuclei, one of which is selected for the egg nucleus while the remaining nuclei migrate back into the vegetative threads. Fertilization occurs by the penetration of a sperm and the fusion of sperm and egg nucleus.

DAVIS (4), working with fixed material of *V. geminata*, studied the fate of the supernumerary nuclei in the oogonia. He claimed that the young oogonia were multinucleate, but that, contrary to OLT-MANNS' observations, the supernumerary nuclei degenerated instead of migrating back into the vegetative thread.

HEIDINGER (6) studied the development of the sexual organs in *Vaucheria pachyderma*, *V. arrhyncha*, *V. terrestris*, and *V. geminata*. In *V. arrhyncha* he followed the development of the sperms and found that those from a single antheridium may take 15-20 minutes to emerge. In all four species he described the supernumerary nuclei as migrating back into the main thread. His work in general corroborates and extends that of OLT-MANNS.

MUNDIE (9) was unable to find the sperms in *V. geminata*, although he killed material quite early in the morning (2:30 A.M.). He thought that perhaps fertilization might be effected by the antheridium and oogonium coming in close contact, a view which has been held also by NÄGELI (10), DIPPEL (5), and KARSTEN (see WALZ 17).

MUNDIE's observations in regard to the fate of the supernumerary nuclei seem, according to his interpretation, to corroborate those of DAVIS, in that he is of the opinion that they degenerate. It is my opinion that MUNDIE's figures, which apparently were drawn with painstaking precision, may be given a different interpretation from that which he has stated. This matter will be discussed later.

Observations

VAUCHERIA SESSILIS

Excellent material of *V. sessilis* in fruit was collected from a small marsh in Chapel Hill, N.C., in January, 1930. This material was kept growing in petri dishes in the laboratory for several days, during which time it bore an abundance of sexual organs. Other lots of material in about the same condition were collected from the same locality several times during the winter and spring of 1930. The sexual organs cease development if the threads are injured to any extent, but by a simple manipulation it was possible to transfer threads to a slide for microscopic observation. Sexual organs in the desired stage of development were located under the low power of the microscope, a cover glass was dropped in the dish by the desired threads, and then under a binocular dissecting microscope with glass needles the threads were dragged carefully through the water on to the cover glass. The cover glass was then lifted out and placed with the material upright on a slide and observed thus without a cover. Material handled even so carefully aborts frequently, and it was found that very satisfactory observations can be made without removing the material from the dish if the threads bearing the sexual organs are gently teased out from the entangling mat.

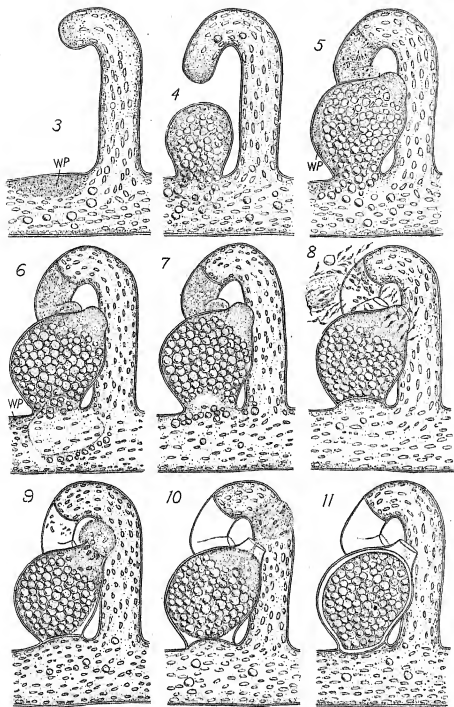
OLTMANN found a very interesting rhythm in the development of the sexual organs in *V. sessilis*, and I have noticed the same phenomenon. This rhythm may be somewhat upset, however, after the material has been in the laboratory for several days. The antheridia start development first and become rather long and curved before the oogonia appear. The antheridia usually start their development in the afternoon, the oogonial branch sprouting in the early part of the night. The following afternoon the oogonial beak appears, the

sperms are discharged, and fertilization occurs sometime during the night, usually after midnight and before 3 A.M.

Since the observations of PRINGSHEIM and OLTMANNS on this species are so detailed, it is unnecessary to repeat their full account except where new or controversial points are concerned.

I have noticed a distinct lashing movement of delicate threads in the antheridial branch a considerable time before the antheridium is cut off. This movement occurs not only in the tip of the antheridial thread in the region which is to become the actual antheridium, but also for a considerable distance down the stalk. In one antheridial branch which was studied particularly on this point, this lashing movement was evident for about two hours before the antheridial wall was formed. These delicate threads extend from the thick cytoplasmic lining out into the vacuoles; rarely one may extend entirely across a vacuole. This movement suggests that the sperms are already forming in the dense cytoplasmic lining. At a later stage, after the cross wall separates the antheridium from the stalk, several (2 or 3) large vacuoles containing many sperms may be noticed, each with two cilia pointed in opposite directions, one of which is still anchored in the cytoplasmic membrane (fig. 5). Shortly before the antheridium opens, the sperms may be seen in vigorous motion, still inclosed in the vacuoles but no longer attached to the cytoplasmic membrane.

In the earliest stages in the development of the oogonia of *V. sessilis*, a very interesting condition has been noticed which has apparently escaped the attention of other observers. Before the main thread has even begun to bulge out to form the oogonial initial, a heaped-up mass of cytoplasm is found near the base of the antheridial stalk. This mass of cytoplasm may sometimes be so large as to extend over one-third of the way across the interior of the thread. It is more or less free from chloroplasts, and in the early stages does not seem to contain nuclei in the region next to the wall, but contains many nuclei in the region toward the center of the thread (figs. 3, 12, 13). This is OLTMANNS' "Wanderplasm" in its earliest stage of recognition. Later, as the thread bulges out, forming the oogonial initial, this mass of cytoplasm occupies the distal growing region, and

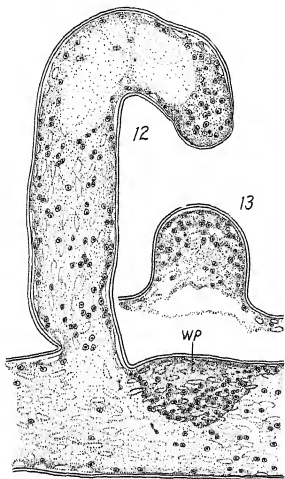


FIGS. 3-11.—Development of sexual organs of *V. sessilis* in living material from shortly after appearance of antheridial branch to mature zygote; observations from April 1 to 6, 1930: Fig. 3, young antheridium and "Wanderplasm" (*wp*), latter in position from which oogonium will sprout, April 1, 9:30 P.M. Fig. 4, young oogonium with peripheral "Wanderplasm," April 2, 8:30 A.M. Fig. 5, oogonial beak formed, "Wanderplasm" retreating into thread; oil globules passing into oogonium; antheridial wall forming, chloroplasts in antheridium degenerating; April 2, 9:30 P.M. Fig. 6, "Wanderplasm" entirely out of oogonium; large vacuole passing in; antheridial wall formed and sperms evident, some free in vacuoles, others imbedded in cytoplasm; April 3, 12:10-12:15 A.M. Fig. 7, basal wall of oogonium forming; sperm very distinct in antheridium; tip of antheridium undergoing hydrolysis; April 3, 12:30-12:40 A.M. Fig. 8, sperms emerging and swarming around oogonial beak. Some swam vigorously for about 14 minutes; together with the sperm, some cytoplasm and oil globules were discharged; April 3, 1:10 A.M. Fig. 9, oogonial membrane broken at tip and cytoplasm exuded; April 3, 1:50 A.M. Note sperms near tip of oogonium; these had crawled about over beak in an amoeboid-like fashion since about 1:25. Shortly after oogonium opened, the sperm disappeared. Fig. 10, cytoplasm extruded from oogonium, rounded up, and separating from egg; sperm apparently entering egg; April 2, 2 A.M. Fig. 11, ripe zygote

stained preparations show that it contains many nuclei (fig. 13). After the oogonial beak is formed, this hyaline area of protoplasm moves to the side of the oogonium opposite the beak, and slowly retreats into the thread (figs.

4, 5).

After the "Wanderplasm" has entirely emerged from the oogonium, a conspicuous vacuole appears in the thread at the base of the oogonium (fig. 6). This vacuole may remain in this position for 10-15 minutes or even longer, or it may pass immediately into the oogonium. A few minutes after the vacuole passes in, vague signs of a basal membrane become visible, and in a few minutes more, as a rule, the basal wall is formed. In the oogonium shown in the accompanying figures, no record was made of the time when the "Wanderplasm" had entirely emerged; the vacuole, however, passed in between 12:10 and 12:15, and by 12:40 the basal wall had been formed. The same sequence of events was observed several times in *V. pachyderma* and *V. aversa*.



FIGS. 12, 13.—*V. sessilis* killed and stained (Gram's method) *in toto*, showing young antheridium and "Wanderplasm." Fig. 12 (combined surface and section view), note "Wanderplasm" by base of antheridium: its outer region is composed of cytoplasm while toward center of thread are vast numbers of nuclei. Fig. 13, oogonial branch sprouting, mostly filled with "Wanderplasm." Section view $\times 625$.

OLTMANN'S does not mention the vacuole, and according to him about an hour elapses between the moment when the "Wanderplasm" has completely emerged and when the basal wall is completed.

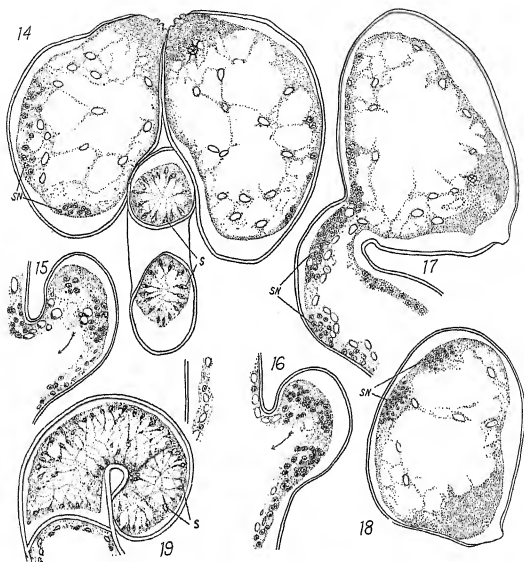
I was unable to get stained sections of the oogonia at this stage to determine whether this retreating protoplasm contained the super-

numerary nuclei, as maintained by OLTMANN. So far as they go, however, these observations clearly substantiate his.

PRINGSHEIM stated that the oogonium and antheridium opened almost simultaneously, the oogonium opening slightly before the antheridium. I have observed the opening of a great number of oogonia and antheridia, and while adjacent ones may open almost simultaneously, it happens just as frequently that either one or the other may open from a few minutes to two hours before the other opens, a condition which would tend to prevent self-fertilization. HEIDINGER describes a sort of intermittent discharge of the sperms in *V. arrhyncha*. This period of discharge from one antheridium lasts from one-quarter to one-half hour. In *V. sessilis* a somewhat similar condition obtains, although the period of discharge does not last so long as in *V. arrhyncha*. It often happens, when the antheridium opens before the oogonium, that some of the sperms, after swimming around the oogonial beak for several minutes, may come to rest on the beak, crawling about over its surface in a somewhat amoeboid fashion. The actual penetration of the sperm into the oogonium has not been observed with absolute certainty in living material, but several stages in this act have been found in fixed preparations.

VAUCHERIA GEMINATA

This species was collected from the same location as *V. sessilis*. In spite of the fact that a considerable number of continuous observations were made on the development of the sexual organs in this species, the discharge of the sperms was never observed. At one time the same antheridium was kept under observation from early afternoon until 5 A.M. the following morning, at which time it was still unopened. In such antheridia the movement of cilia-like structures was observed, and great numbers of completely empty antheridia were seen. In material killed about 3 A.M. and stained *in toto*, or sectioned and stained (iron-alum haematoxylin or Gram's gentian violet method), the sperms could be clearly seen (figs. 14, 19s). In the sectioned material the sperms were arranged exactly as described by OLTMANN for *V. sessilis*; that is, one of the ends of the sperms was fastened in the cytoplasmic layer, and the free ends, each with a single cilium attached, extended out into the vacuole (fig. 14). In



FIGS. 14-19.—*V. geminata*: Fig. 14, horizontal section through two oogonia and one antheridium, showing enlarged female gamete nucleus in oogonium to right; female gamete nucleus also present in oogonium on left but not in section drawn. Note supernumerary nuclei (sn) and degenerating chloroplasts, which might be confused with nuclei in poorly stained preparations, scattered throughout the cytoplasm. Two sections of antheridium shown, both with distinct sperms with one end fastened in cytoplasmic membrane. Fig. 15, showing stalk of oogonium on right, and clusters of nuclei which have passed out of oogonium. Fig. 16, stalk of same oogonium nearer thread, and showing vast numbers of nuclei migrated from oogonium. Fig. 17, section through oogonium and stalk showing female gamete nucleus. (Many oogonia were seen in this condition with the female gamete nucleus invariably in this position.) Note large number of nuclei which have migrated from oogonium. Fig. 18, section of same oogonium as shown in fig. 17, showing clusters of nuclei; very few nuclei showed signs of degenerating. Fig. 19, antheridium stained *in toto* with iron-alum haematoxylin; sperm not so far developed as in fig. 14. All $\times 625$. All material killed 3 A.M.

the sketch of the entire antheridium (fig. 19) the sperms are not so advanced in their development as shown in figure 14. My failure, as well as MUNDIE'S, to observe the discharge of the sperms in *V. geminata* was doubtless due to insufficient observations.

Because of the position of the oogonia, it is very difficult to follow the "Wanderplasm" in this species in living material. Several cases were seen, however, which showed a very distinct "Wanderplasm" apparently passing from the oogonium back down the thread.

In fixed material and preparations stained *in toto*¹ a number of oogonia were found which showed clearly that most of the nuclei pass back into the threads, but no single oogonium was found which did not still contain a considerable number of nuclei. In such oogonia the supernumerary nuclei are disposed near the wall, almost invariably being arranged in clusters (figs. 14, 18). Although great numbers of oogonia have been examined at this stage, the nuclei have never been found scattered throughout the cytoplasm as figured by DAVIS. In several slides there were sections of *V. terrestris* showing well stained nuclei scattered through the cytoplasm somewhat as shown in DAVIS' figures. DAVIS shows only 9-15 nuclei in his sections of oogonia, whereas in sections which show the "Wanderplasm" well the number of nuclei is much larger. In some oogonia many of these supernumerary nuclei are peripherally disposed, and are arranged in such a way as to be apparently going to take part in the formation of the egg wall. Such nuclei often show various stages of degeneration. Unfortunately, since all of the material was killed at 3 A.M., all of the oogonia which are developing are in almost exactly the same stage. All show a single, enlarged egg nucleus in the

¹ The method used in preparing fixed material of both *V. geminata* and *V. aversa* for study was as follows: Kill in Chamberlain's chrom-acetic solution for *Vaucheria* 6 hours; wash 24-48 hours in still water; dehydrate with 5-100% alcohols; transfer to xylol $\frac{1}{4}$ -alcohol $\frac{3}{4}$ 30 minutes, xylol $\frac{1}{2}$ -alcohol $\frac{1}{2}$ 30 minutes, xylol $\frac{3}{4}$ -alcohol $\frac{1}{4}$ 30 minutes, xylol 30 minutes; then transfer to xylol and paraffin, then paraffin 2 hours; imbed in 58° paraffin. Section 6-10 μ thick; clear; descend in alcohols to water; stain in aniline gentian violet 10 minutes; dip several times in water to remove surplus stain; treat with iodine-potassium iodide solution 2-5 minutes; dip several times in 95% alcohol (not over 30 seconds), 100% alcohol 1-2 minutes, clove oil (flood slide 2 or 4 times), cedar oil, xylol 5 minutes; and mount in balsam.

For more complete details on GRAM's method, see COUCH, J. N., The development of the sexual organs in *Leptolegnia caudata*. Amer. Jour. Bot. 19:584-599. 1932.

dense region of cytoplasm at the beak. The egg nucleus, however, is invariably in the margin of the cytoplasm toward the center of the oogonium (figs. 14, 17). MUNDIE (figs. 2, 4, 11, 14) shows the female gamete nucleus in about this position. One of his figures, however, shows the nucleus rather close to the tip (MUNDIE, fig. 13). DAVIS describes and figures the female gamete nucleus in the center of the egg. Since the basal wall had not been formed in any of these oogonia, it is not possible to say what is the ultimate fate of the supernumerary nuclei which are still in the oogonium at this time.

MUNDIE killed his material at 2:30 A.M., while mine was killed at 3 A.M. It is interesting that the oogonia in his material and in mine should have been so nearly in the same stage of development. MUNDIE finds the supernumerary nuclei in the oogonia in much the same position as here described, that is, they are in the position where the "Wanderplasm" is supposed to be. He also shows a few nuclei back in the stalk which may have migrated from the oogonia. These nuclei in the stalk are so abundant and conspicuous that, if the material is killed at the right time and properly fixed and stained, one cannot fail to observe them (figs. 15-17). It is probable that most of the supernumerary nuclei figured by MUNDIE would have passed out of the oogonia if his material had been killed an hour or so later.

There seems to be considerable confusion as to the time when the cross wall is formed. DAVIS, working entirely with fixed material, states that the wall appears when the oogonium is about two-thirds its mature size, while MUNDIE says that the cross wall is not formed until after fertilization. Unfortunately I cannot say precisely when the cross wall is formed in this species. Examination of a great number of longitudinal sections through the oogonium and stalk, as in figure 17, indicates that the basal wall is not formed until a considerable time (several hours) after the oogonium attains its mature size. Of course, if the basal wall were formed when the oogonium is only two-thirds mature in size, as claimed by DAVIS, the supernumerary nuclei would be held in the oogonium and could not retreat into the thread. The migration of the "Wanderplasm" does not begin in *V. sessilis*, *V. pachyderma*, and *V. aversa* until the oogonia have reached mature size, and it is probable that the same sequence of events occurs in *V. geminata*. MUNDIE also is almost certainly wrong as to

the time when the basal wall is formed. His figures 11 and 14, intended to show stages in fertilization, are almost certainly some considerable time before that act. The oogonia have not opened at the tip, an occurrence which invariably precedes the entrance of the sperms in all other species which have been investigated; nor does either of the oogonia (MUNDIE's figures 11 and 14) show any sort of perforation in the wall through which the sperm substance might have been transferred. It is very probable that the basal wall in *V. geminata* is formed in much the same way and at about the same time (about an hour or so before the oogonium opens) as in *V. sessilis*, *V. pachyderma*, and *V. aversa*.

VAUCHERIA PACHYDERMA

In *V. pachyderma* there is considerable "Wanderplasm," and hence this species is one of the best for observations on the fate of that substance. On March 25, 1930, and for several days thereafter, excellent fruiting material of *V. pachyderma* was found growing on a damp bank in the arboretum at Chapel Hill, N.C. Several continuous series of observations on the development of the oogonium and antheridium were made, all with essentially the same results. On the first series observations were begun at 4 P.M., and except for about 45 minutes' absence were continuous until 4 A.M. Twelve camera-lucida sketches were made at intervals of about an hour to show the changes through which the oogonium and antheridium passed. Four of these sketches are reproduced in figures 20-23.

The oogonium and antheridium, selected for observation (fig. 20), were mature in size at 4 P.M., when the observations were begun. Both organs were still in communication with the main thread, although signs of a membrane separating the more or less colorless contents of the antheridium from its stalk had become apparent. The antheridium contained granular cytoplasm in the center of which was a large vacuole. Close to the membrane delimiting the antheridial cytoplasm from that of the stalk were numerous disintegrating chloroplasts. There were also a few scattered here and there through the antheridial cytoplasm. The antheridium contained little or no oil.

The oogonium had developed a distinct beak which contained

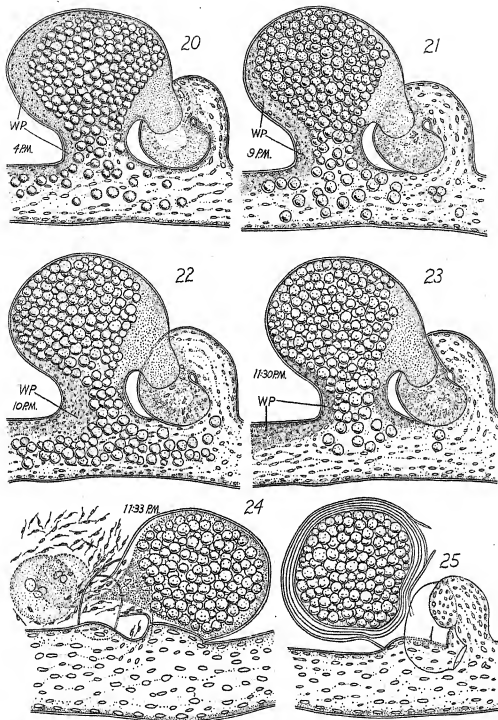
colorless cytoplasm. Two or three rather distinct granules, which were sometimes apparently furnished with astral rays, could be seen in the oogonial beak. The central part of the oogonium was apparently occupied by a large vacuole. On the sides of the oogonium, oil globules were collected in great numbers. At the back, opposite the beak and extending from the top down to the neck, was a dense layer of granular cytoplasm containing a very few scattered chloroplasts (fig. 20). This dense layer of cytoplasm is the "Wanderplasm" of OLTMANN and HEIDINGER.

At 8 P.M. the membrane separating the antheridium from the antheridial stalk had moved up considerably. A small vacuole had appeared near the tip of the antheridial cell, and a faint motion of cytoplasmic threads was visible in the vacuole. No perceptible change had taken place in the oogonium, except that the "Wanderplasm" had become thicker toward the base and in the neck. Attention was now concentrated on the "Wanderplasm," and immediately it was seen to be moving slowly outward into the stalk. This movement was exceedingly slow and was alternated with periods of apparent complete quiescence. The movement was best detected by focusing attention on one granule as it passed through the neck. By 9 P.M. the "Wanderplasm" had receded entirely from the top of the oogonium and was continuing to move out into the thread (fig. 21).

At this time three or four elongated rods could be seen moving autonomously about in the vacuole in the antheridial cell. These were evidently precocious sperms. Rodlike bodies were also becoming apparent here and there in the cytoplasm of the antheridium.

By 9:30 P.M. the "Wanderplasm" had moved out of the oogonium in such quantity that it occupied more than one-third of the space in the neck. This mass was divided into two rather distinct zones: an outer zone of pale green, finely granular cytoplasm and an inner zone of still paler green, hyaline cytoplasm with large granules. In the antheridium the vacuole had become somewhat more distinct, and now contained several elongated motile bodies.

At about 10 P.M. approximately half the space in the neck was occupied by the "Wanderplasm" (fig. 22). The movement continued, until by 12:30 A.M. the entire mass has passed from the oogonium back into the thread. At about 11 P.M., when the "Wander-



FIGS. 20-25.—*V. pachyderma*: Fig. 20, conspicuous "Wanderplasm" on top, back, and extending down into thread. Oögonium contains much oil; beak filled with clear cytoplasm; antheridial wall forming. Antheridium contains granular cytoplasm and degenerating chloroplasts; 4 P.M. March 26, 1930. Fig. 21, "Wanderplasm" receding from oögonium; sperm evident in antheridium; 9 P.M. March 26. Fig. 22, "Wanderplasm" mostly receded from oögonia; 10 P.M. March 26. Fig. 23, "Wanderplasm" practically entirely out of oögonium and oil globules passing into oögonium; 11:30 P.M. March 26. Fig. 24, antheridium and oögonium which opened almost simultaneously; 11:30 P.M. Fig. 25, oögonium with ripe zygote. All $\times 325$.

plasm" had almost entirely receded from the oogonium, the oil globules in the thread were observed to be moving into the oogonium, the passage inward continuing until most of the oil which had accumulated in this region of the thread had moved into the oogonium (fig. 23). Between 11:30 and 12 the membrane between the antheridium and its stalk was replaced by a distinct wall. At this time close observation would reveal the sperms imbedded in the cytoplasm. The antheridium also still contained a few pale green chloroplasts. By 1:15 A.M. numerous sperms could be seen, both in the cytoplasm and in the vacuoles of the antheridium. About 1:15 A.M. a large vacuole passed from the thread into the oogonium, and almost immediately a membrane between the oogonium and its stalk became evident. This same sequence of events was observed in several other oogonia. No apparent noteworthy change took place in the oogonium and antheridium between 1:15 and 3 A.M., except that the vacuole changed its shape somewhat and the basal membrane of the oogonium became more distinct.

At 3:10 A.M. some fresh water was added to the *Vaucheria*, and in a few minutes the antheridium opened. Instead of discharging motile sperms, the contents of the antheridium slowly oozed out into a mass which assumed a spherical shape resembling the undifferentiated, discharged content of a *Pythium* sporangium. This mass of material consisted of undifferentiated sperms, cytoplasm, and a number of differentiated sperms some few of which succeeded in freeing themselves from the mass. The bladder containing the sperms remained at the tip of the antheridium until after 3:30 A.M., about which time it became separated from the tip and sank to the bottom of the dish. The oogonium had not opened at 4 A.M., at which time observations were discontinued. The following morning the oogonium still had not opened, but contained a mass of large oil globules.

In several other series of observations the normal opening of the antheridium and oogonium was observed. Suddenly the tip of the oogonial beak gives way and the hyaline cytoplasm begins to ooze out. The cytoplasm continues to stream out for one or two minutes, forming at first an elongated twisted mass. While this is coming out the antheridium usually bursts. In a few seconds more the mass of cytoplasm extruded from the egg assumes a rounded shape, becom-

ing separated from the long tapering strand of cytoplasm which extends out from the egg. Meanwhile the sperms are swarming about the mass of cytoplasm which has been extruded from the egg, and great numbers of them swim about the oogonial tip, some entering the space between the egg and oogonial wall (fig. 24). As soon as the extruded sphere of cytoplasm becomes separated from the egg cytoplasm, the protruding tip is drawn back into the egg. A membrane now forms around the egg and the latter swells considerably, completely filling the oogonium. Sometimes the egg may continue to swell so that another mass of cytoplasm is extruded, after which the tapering tip is drawn back into the egg, the tip rounding off as before. This has been observed three times in succession in the same oogonium.

I have been unable to observe a sperm actually enter the egg. Apparently this would most likely occur while the egg is in the condition shown in figure 24. The sperms remain active for only a short time (about 10 minutes) in this species. After a few days a thick wall is formed around the zygote and it becomes separated from the main thread (fig. 25).

VAUCHERIA AVERSA

LIVING MATERIAL.—This species affords even more favorable material for studying the development of the sexual organs than *V. pachyderma*, particularly in regard to the development of the antheridium, since in *V. aversa* this organ is straight and is so placed that it is seldom obscured by the oogonium. This species also has a large quantity of "Wanderplasm," a characteristic facilitating observations on the fate of that substance.

Vaucheria aversa is rather common, although not abundant, around Chapel Hill, N.C., occurring from January to April in still or running water or on damp soil.

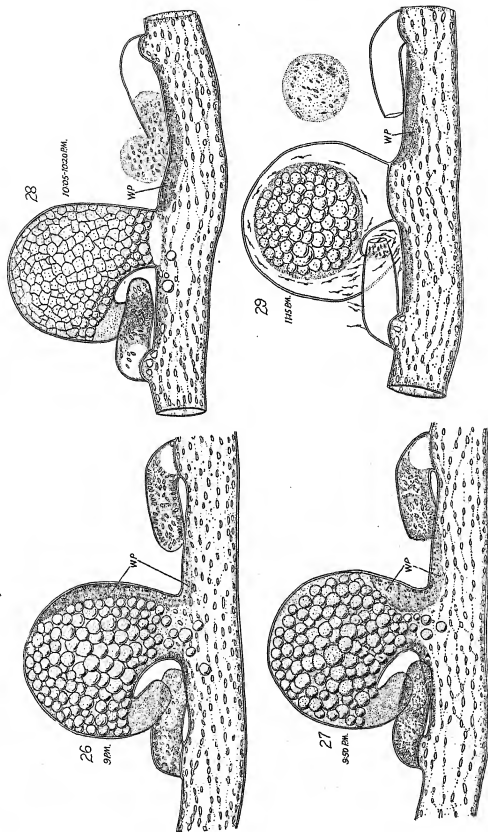
In this species the early development of the sexual organs was not studied, but observations were begun in the late afternoon or early evening on oogonia and antheridia which had reached mature size. Such an oogonium with its accompanying antheridia is shown in figure 26. The oogonium is comparatively very large, with a distinct beak which curves downward and inward toward the base of the oogonium. Each oogonium almost invariably has two antheridia,

both arising from the main thread, one in front of the oogonium and the other behind, and both extending toward the oogonium. The oogonium at this time (9 P.M.) contained many large oil globules which were mostly on the sides against the wall. The beak contained colorless cytoplasm, and toward the back there was a layer of more or less colorless cytoplasm which extended from the top down into the thread for some distance. This is the "Wanderplasm" of OLTMANN and HEIDINGER. The central part of the oogonium was apparently occupied by a vacuole. At this stage of development both antheridia contained distinct sperms, most of which were imbedded in the peripheral cytoplasm.

By 9:50 P.M. the vacuole in the oogonium had increased in size and the "Wanderplasm" had mostly emerged from the oogonium. By focusing attention on the latter its slow movement out of the oogonium could be seen (fig. 27). Fifteen minutes later the "Wanderplasm" had completely emerged from the oogonium and the basal wall was beginning to be formed. The "Wanderplasm" was still evident out in the thread (fig. 28). The colorless cytoplasm in the beak region had increased in area. At this stage it appeared that the internal pressure in the oogonium was considerable, for the oil globules which heretofore had been more or less rounded now pressed against one another so that they appeared polygonal in shape. The beak also showed the effect of this pressure, straightening out somewhat.

At 10:05 one of the antheridia opened and the contents began slowly pouring out and forming a spherical mass at the tip of the antheridium. The contents passed out so slowly that it took 45 minutes for the antheridium to become completely empty. During this time a faint movement of the sperms was evident. At 11:12 the mass separated from the antheridium and sank to the bottom of the dish. At this time the sperms had become rather active in the mass. Several antheridia were seen to discharge in the same way as in *V. pachyderma* and *V. sessilis*.

About 11:15 the antheridium near the oogonial beak opened and the entire contents poured out in a few seconds (fig. 29). Simultaneously the oogonial beak burst and the contents shrank from the wall, rounding up in the center to form the egg. The sperms entered in



FIGS. 26-29.—*V. acroa*, showing fate of "Wanderplasm" and development and discharge of sperms. Fig. 26, "Wanderplasm" receding from oogonium; sperms evident in antheridium. Note large vacuole at base of antheridium at right; 9 A.M. April 6, 1930. Fig. 27, same oogonium 50 minutes later; very conspicuous "Wanderplasm" almost out of oogonium which contained large vacuole not shown in figure; sperm becoming more distinct. Fig. 28, same oogonium 15-30 minutes later; "Wanderplasm" entirely out but still visible in thread. Pressure in oogonium causing flattening of oil globules; basal wall of oogonium forming. Contents of antheridium on right passing out as in sporangia of *Pythium*; sperms distinct. Fig. 29, same oogonium 45 minutes later. All $\times 325$.

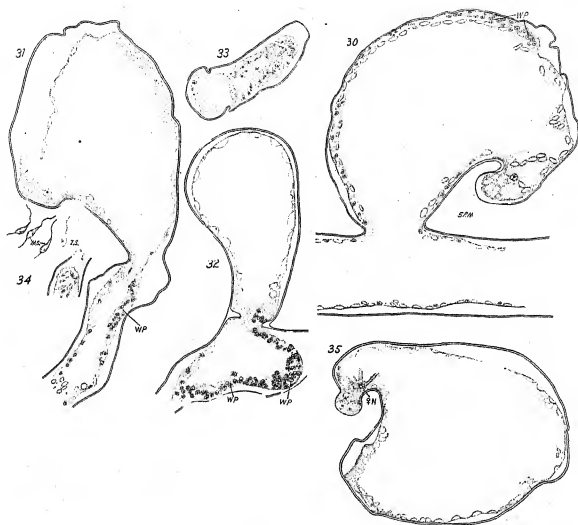
great numbers, swarming about the egg. I was unable, however, to see a sperm enter the egg.

FIXED MATERIAL.—Oogonial material of *V. aversa* was killed in Chamberlain's solution for *Vaucheria* at 5 and 11:30 P.M., April 6, 1930. Sections were cut 6 and 8 μ thick and stained with an anilin solution of gentian violet and a solution of orange G in clove oil.

Sections of oogonia killed at 5 P.M. showed two very instructive conditions in regard to the fate of the "Wanderplasm." Several sections of oogonia were seen just before the oogonial beak was well formed and before the egg nucleus had become evident. In such oogonia there was a large central vacuole surrounded by a rather thick cytoplasmic layer. Numerous nuclei were scattered more or less evenly throughout the cytoplasm. Sections of other oogonia killed at the same time showed considerably later stages of development. Such a section is shown in figure 30. The oogonium had attained its mature size and the beak process had been formed. The beak was almost full of rather dense cytoplasm, in the center of which was a comparatively large nucleus, the female gamete nucleus. The central vacuole had increased greatly in size with a simultaneous thinning of the peripheral cytoplasm. The most remarkable change between this oogonium and the one just described, however, was in the position of the nuclei, which were located near the beak region. Immediately after the selection of the egg nucleus, the supernumerary nuclei began to recede from the region of the beak. This is strikingly shown in figure 30. In this oogonium, all the sections of which were well fixed on the slide, all the supernumerary nuclei had migrated from the beak region, passing backward toward the top of the oogonium. In this region there were many nuclei.

The supernumerary nuclei continued slowly to migrate backward from the oogonium until none but the female gamete nucleus was left. It appears as though the supernumerary nuclei are actually repelled by the female gamete nucleus. Material killed at 11:30 P.M. showed oogonia from which all the "Wanderplasm" with its contained nuclei had passed (figs. 31, 32). The number of nuclei which pass out is enormous. A count was made of the number which emerged from one oogonium and the number which appeared in four sections was 250. Two of the sections near the center were

lost. Allowing 75 nuclei for each of these sections, the total number would be about 400. This large number was perhaps due to the fact that the nuclei underwent almost simultaneous mitosis as they



FIGS. 30-35.—Fig. 30, section of oogonium of *V. aversa* showing egg nucleus and tip region of oogonium from which supernumerary nuclei have receded, accumulating toward top of oogonium (*wp*); killed 5 P.M. $\times 625$. Figs. 31 and 32, sections of same oogonium from which nearly all the supernumerary nuclei (*wp*) have passed. Note egg nucleus in tip and scant amount of cytoplasm left in oogonium. Figs. 33 and 34, sections of the two antheridia accompanying this oogonium showing very distinct sperms; killed 11:30 P.M. All figures except free sperm in fig. 34, $\times 625$; free sperm, $\times 1000$. *ms*, mature sperm; *is*, immature sperm. Nucleus in immature sperm more distinct than in mature sperm. Fig. 35, oogonium with basal wall formed, supernumerary nuclei having all passed out; female gamete nucleus and few chromatin granules in tip, perhaps the remains of degenerated nuclei. $\times 625$.

emerged. When the "Wanderplasm" has all emerged from the oogonium, a very scant amount of cytoplasm is left (fig. 35). My material, unfortunately, did not show stages in fertilization.

The emergence of the supernumerary nuclei, together with some of the cytoplasm, or "Wanderplasm," from the oogonia in *Vaucheria* is indeed one of the most remarkable phenomena yet described in the oogenesis of any of the Thallophytes. In fact it is so unusual that neither of the Americans (DAVIS, MUNDIE) who have worked on the cytology of *Vaucheria* have been willing to accept the evidence offered by OLTMANNS and HEIDINGER. It is easy to observe the fate of the "Wanderplasm" in *V. sessilis*, *V. pachyderma*, or *V. aversa* simply by following through the development of the oogonia in living material. Indeed since the development usually follows a definite rhythm, it is essential first to study living material in order to discover this rhythm, so that one may fix material in the desired stages of development. OLTMANNS, who first noticed this rhythm in the development of the oogonia, described it in great detail in his classic paper. Neither DAVIS nor MUNDIE appears to have made any continuous observations on living material, or to have known of the rhythmic development described by OLTMANNS, and this probably explains why they failed to observe the "Wanderplasm" in *V. geminata*.

So far as I have been able to discover, the only other plant which shows this peculiar economy in preserving the supernumerary nuclei, or part of them, during gametogenesis is *Endogone lactiflua*. In this fungus, as described by BUCHOLTZ (3), the large gametangium is multinucleate, containing one large gamete nucleus and many smaller ones. Before the basal wall is formed all or the greater number of the small supernumerary nuclei migrate back into the thread. The supernumerary nuclei which do not withdraw into the thread degenerate.

Summary

1. A survey of the available literature on sexual reproduction in *Vaucheria* from VAUCHER down to the present time is given. From this survey it appears that the sperms have been observed in all species investigated, with the possible exception of *V. geminata* (there is some doubt about the plant studied by SCHENK being *V. geminata*).

2. The development of the sperms and their discharge were ob-

served in *V. sessilis*, *V. pachyderma*, and *V. aversa*. In *V. geminata* the development of the sperms and their movement in the antheridium were observed, but the discharge of the sperms was not seen. In the species in which discharge was observed this usually occurred at night, sometime after midnight. The present observations on the development of the sperms corroborate those of PRINGSHEIM, WALZ, WORONIN, OLTMANN, and HEIDINGER.

3. In *V. sessilis* the "Wanderplasm" initiates the formation of the oogonial branch, but as soon as the oogonium is about mature the "Wanderplasm" migrates back into the main branch.

4. In *V. geminata* the "Wanderplasm" passes back out of the oogonium, carrying most of the supernumerary nuclei. Although observations are incomplete, it appears that some of the nuclei may remain in the oogonium to degenerate.

5. In living material of *V. pachyderma* there is a large and conspicuous "Wanderplasm" which passes entirely back into the main thread.

6. Studies on living material of *V. aversa* gave essentially the same results as in *V. pachyderma*. In *V. aversa* stained material showed with apparent certainty that practically all of the supernumerary nuclei migrate back into the main thread with the "Wanderplasm."

7. In *V. sessilis*, *V. pachyderma*, and *V. aversa* the basal wall of the oogonium is formed from 15 minutes to an hour after the "Wanderplasm" has passed out, and from a few minutes to an hour before the oogonium opens.

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FURTHER STUDIES ON COLD RESISTANCE IN EVER- GREENS, WITH SPECIAL REFERENCE TO THE POSSIBLE RÔLE OF BOUND WATER¹

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Introduction

With few exceptions, the exposed portions of all plants living in habitats where temperatures fall below freezing at certain periods of the year must possess the property of cold resistance,² at least during the actual duration of such periods of subzero temperatures. The only portion of such plants which is normally not exposed to the prevailing atmospheric temperatures is the root system, and usually only a part of it escapes freezing temperatures. In some species the interior of massive structures such as tree trunks may also escape exposure to temperatures as low as those which may be reached in other parts of the same plant, owing to temperature lags. In many latitudes certain species of plants and parts of other species are blanketed under heavy snowfalls for most or all of the cold period of the year. Such plants or plant parts probably often do not develop the degree of cold resistance possessed by those exposed directly to low temperatures. Submerged water plants are another group in which the property of cold resistance, as the term is used in this paper, probably often does not develop.

In species which retain their leaves during the colder months of the year, the leaves as well as the exposed woody parts of the plant must possess this property of cold resistance. Such evergreen leaves are not, in general, resistant to cold during the warmer months of the year. The writer's experiments conducted with leaf-bearing branches of the pitch pine (*Pinus rigida* Mill.) have shown invariably that if they were exposed for periods of several hours to a tempera-

¹ Papers from the Department of Botany, the Ohio State University, no. 301.

² The term cold resistance as used in this paper refers only to the ability of plant tissues to survive unharmed periods of freezing temperatures which ordinarily would be expected to kill or severely injure them, owing to the formation of ice crystals. The term hardness as usually applied to plants has essentially the same connotation.

ture of -20° C. during the winter months no damage resulted. Similar branches collected during the summer months and similarly exposed invariably showed a complete or nearly complete killing of the leaves on those branches. Similar results with other evergreens are described in detail by WINKLER (32). Evidently the property of surviving unharmed periods of freezing weather is possessed by evergreen leaves in general only during those periods of the year when such temperatures frequently prevail.

Apparently the leaves of evergreens such as the pitch pine go through an initial "hardening" process during the autumn months, and a corresponding final "dehardening" process during the spring months. In the latitude of Columbus, as an earlier investigation by the writer (17) has shown, this hardening process usually takes place during November, while the corresponding dehardening process usually occurs in May.

The preceding statements should not be understood to preclude the possibility that dehardening and hardening processes may also occur during the winter months. WINKLER, for example, found that the leaves of a number of evergreen species, all of which on January 2 withstood a temperature of -18° C. without injury, were practically all killed if branches of these evergreens were kept in a room at 20° C. for 16 days and then exposed to a temperature of -12° C. According to the work of TUMANOV (30), wheat plants lose a relatively large part of their cold resistance very quickly upon being brought into a warm room. For example, wheat plants hardened at 7° C. for 5 to 7 days lost nearly half of their relative cold resistance upon being kept in a room at 15° – 17° C. for one day. The results of both of these investigations show that during warm periods in the winter a partial or complete loss of the ability to withstand temperatures below freezing occurs very commonly, at least in some species. With the onset of cooler weather, the leaves of such species again become hardened.

In another experiment TUMANOV showed that as short a period of exposure as one day to a temperature of 5° C. markedly increased the cold resistance of wheat plants, although maximum resistance developed only with longer periods of exposure (two to three weeks). In this species the plants pass out of the condition of hardiness much more rapidly than they gain it. The hardening experiments of HAR-

VEY (10), ROSA (24), and others also showed that the condition of hardiness may be rapidly established in plants under the influence of cool temperatures.

The writer has, so far at least, been unable to find any evidence of any appreciable loss of cold resistance by the leaves of the pitch pine during the winter months. In one experiment, branches of this species were brought into the greenhouse in February and kept with the cut ends in water for a period of three weeks. Parts of these branches were then exposed to a temperature of -20°C . for several hours without any resultant injury to the leaves. Several similar experiments gave practically identical results. It appears possible that species may vary decidedly in the property of losing and regaining hardiness during the winter months. Perhaps this is related to a dormancy factor. The pitch pine is a species in which winter dormancy is very profound. However, the number of experiments which have been performed on this point is still limited, and the conclusion that warm weather will not diminish the cold resistance of pitch pine leaves during the winter months must still be regarded as tentative.

The work of HARVEY (9, 10), TUMANOV (30), and others indicates that the threshold temperature for inducing hardiness or cold resistance in plants is about 5° – 6°C . HARVEY (10) found that exposure of cabbage plants for a period of one to four hours per day to a temperature of 0°C . kept these plants in a condition of hardiness, even if they were exposed to temperatures of 10° – 20°C . during the remainder of the day. This would appear to indicate, as he suggests, that as long as outdoor temperatures fall to a few degrees above zero for a short period each day, plants will remain resistant to injury at much lower temperatures. According to this conception, cold resistance would be induced in evergreen plants during the autumn months as soon as a fairly consecutive period of days occurred during which the temperature dropped close to 0°C . for a few hours each day. As late into the spring as such meteorological conditions persist plants would remain resistant to cold. Some species apparently may lose their cold resistance rather rapidly if a pronounced warm spell occurs during the winter months. Such species normally become hardy again as soon as exposed to hardening temperatures. On the other hand, there may be some plants which main-

tain their cold resistance, even through comparatively warm spells during the winter months. It is believed, as previously noted, that the pitch pine belongs in this class.

It is not proposed in the present paper to give any general discussion of the several important theories regarding the mechanism of low-temperature injury in plants, nor of the various theories of cold resistance in plants. Excellent résumés of the literature on these topics have been made by several previous investigators in this general field. The publications of CHANDLER (4), HARVEY (9), ROSA (24), NEWTON (21), HILDRETH (11), ÅKERMAN (1), and others contain excellent bibliographies of this subject. The most recent critical review of the work in this field is that of MAXIMOV (15).

The results of a previous investigation (17) upon the factors involved in the cold resistance of pitch pine leaves appeared to justify several general conclusions. No correlation could be found between the total water content of the leaves and the phenomenon of cold resistance in this species. The maximum difference between the water contents of hardened leaves during the winter and unhardened leaves during the following summer was found to be less than 2 per cent. Even when calculated in terms of hydration of the dry matter, this difference is relatively small. In actual fact the minimum leaf-water contents were found to occur in the late spring, at a period when the leaves of this species in Ohio are seldom subjected even to 0° C. temperatures, and never to appreciably lower ones. A marked accumulation of soluble carbohydrates was found to occur in the leaves during the winter months. Associated with this was an increase in the osmotic concentration of the expressed leaf sap of approximately equal magnitude. This led to the belief that the winter increase in osmotic concentration of the leaf cells is due largely to increase in soluble carbohydrates. Finally, on the basis of pressure dehydration experiments, it was concluded that an accumulation of colloidal water-imbibing substances occurred in the leaf cells of this species during the winter months, resulting in an increased imbibition pressure in the leaf cells. The increase in soluble carbohydrate content of the sap and the apparent increase in water-retaining capacity of the leaf tissues were considered to be the two most important internal conditions correlated with the property of cold resistance in

the leaves of this species. A correlation between accumulation of carbohydrates and cold resistance in plants has been traced by many previous investigators, and it is not the purpose of this paper to pay further attention to this phase of the problem of cold resistance. The investigations of ROSA (24), NEWTON (20, 21), MARTIN (14), STRAUBAUGH (27), MEYER (17), and DUNN and BAKKE (6) especially have contributed to the conception that an increase in the proportion of water-retaining substances or of "bound" water in plant cells is an important factor in cold resistance. The contributions of these workers will be considered in more detail later in the discussion. In the present paper further consideration is given to the hypothesis that the accumulation of water-retaining colloids, or an increase in the bound (unfreezable) water, is an important factor in the cold resistance of the leaves of the pitch pine, and presumably of other evergreens.

Methods

The pine needles used in this investigation were obtained from native pitch pine trees which were growing on a hill slope in Fairfield County, Ohio, at a point about 35 miles southeast of Columbus. The pines in this location are about 20 years old and compose a rather open but otherwise typical "old-field" stand. For each set of determinations one branch was cut from each of eight different trees. Branches which were similar in exposure and general appearance were chosen. The material was collected late in the afternoon; after being transported to Columbus it was kept at out-of-doors temperatures overnight. The pine leaf samples were collected on August 25, 1931, and on January 15, 1932. The January collection was made just after a period of more than a week during which freezing or nearly freezing temperatures were attained every day. Even if the leaves of the pitch pine do lose their hardness during winter warm spells, which seems improbable, conditions for some time just preceding this collection of leaves were favorable to the development of the hardened condition. For each collection determinations were made the next day.

Determinations were made on each of the eight branches of each collection of the total water content, resistance to dehydration under pressure, and amount of water which could not be frozen at -20°C .

(bound water). About 100 gm. of the 1931 crop of leaves were picked from each branch. All dead leaves and all dwarf branches were removed. Twenty gm. of each batch of leaves were used for the water-content determination. A second 20-gm. portion was used in the bound water determination, while about 50 gm. were used for the pressure dehydration determination.

The water-content determinations were made by drying the leaf samples to constant weight *in vacuo* at 70° C.

The determinations of the resistance of the leaves to expression of sap under pressure were made in a press chamber of a type described previously (18). The samples were first ground, and the ground sample weighed. Each was then subjected to a series of increasing pressures, ranging up to 3000 pounds per square inch, and the amount of sap which had been expressed when the tissue came to equilibrium with each pressure was recorded. From these data, and from the data on the total water content of the leaves, it is possible to calculate the hydration of the leaf tissue at each pressure and, if desired, to plot the actual pressure dehydration curves.

A determination of the amount of water in organic tissues which will not freeze at -20° C. has been considered by a number of investigators (2, 8, 23, 25, 26, 29) to be a measure of bound water. This quantity will be so referred to in this paper, although not necessarily with any specific implications regarding the nature of this bound water. Evidently, however, such water is in a special physical state; very commonly it is considered to be water which is held on surfaces by adsorption forces of sufficient magnitude to prevent the crystallization of water at this temperature. This unfreezable or so-called bound water may sometimes represent, in plant tissues at least, simply that portion of water in a solution which remains unfrozen at any given temperature below 0° C. At any temperature down to the eutectic point a certain portion of the water in a solution will remain liquid. This is due to the vapor pressure equilibrium which is maintained between the ice and the solution at temperatures below zero. As the temperature falls the concentration of the solution increases, owing to ice formation, until its vapor pressure equals that of the ice. This increase in the proportion of water in the frozen state continues until the eutectic point is reached, at which temperature the

solute crystallizes out and all of the water is converted into ice. Of course in solutions which are initially relatively dilute, the proportion of the water which remains liquid at -20° C. is very small.

The amount of bound water was determined by the calorimetric method. Such a method apparently was first used, in a rather crude form, by MÜLLER-THURGAU (19). Later a much improved technique was proposed and employed by RUBNER. RUBNER's work became more widely known through the subsequent investigations of THOENES (29). Further refinements in this method have been introduced by ROBINSON (23), ST. JOHN (25), and SAYRE (26). The method about to be described, although modified to suit the present investigation, follows in general the work of these investigators. Since the experimental part of this work was completed, important papers dealing with certain theoretical aspects of bound water and its measurement by BRIGGS (2) and by JONES and GORTNER (13) have appeared. These papers will be referred to in more detail later in the discussion.

The pine leaves as used in the bound water determination were cut once into half, but otherwise were left intact. Each 20-gm. sample was inclosed in a brass freezing tube 1 inch in diameter and 6 inches in length. Each tube was stoppered at each end with a tight-fitting rubber stopper after the sample had been placed within. The stopper which was inserted in the top end of the tube carried a thermometer, set at such a level that its bulb was approximately in the center of the mass of leaves. These tubes were immersed vertically in an electrically refrigerated low temperature bath³ in which a temperature of -20° C. was maintained.

The freezing tubes were allowed to remain in this bath for several (3-4) hours, temperature equilibrium between the sample and the bath being reached some time before they were removed. Checks

³ This bath was adapted from a General Electric water-cooler. The bottle was removed and the chamber of the cooler, which is about 10 inches deep and has an 8-inch top inside diameter, was nearly filled with 50 per cent alcohol. The switch was set so that the cooler would run continuously. It was supplied with a lid to support the tubes with the thermometers protruding, and a stirring device to keep the temperature uniform throughout the bath. This has proved to be an excellent device for small-scale experiments where temperatures down to -20° C. are required. If necessary, such an arrangement can be provided with a thermostatically controlled heating element, but this was found to be unnecessary in the present work.

with different time intervals indicated that the freezing of water in the samples attained an equilibrium value in this period. BRIGGS (2) also records that with various organic colloids, as much ice formed at -20°C . in 3-4 hours as in a week, indicating that equilibrium was attained in the shorter period of time. JONES and GORTNER (13) state that in a 50 per cent gelatin gel, freezing equilibrium is attained in less than one hour at -21°C . At -11.1°C ., five hours were required for the attainment of this equilibrium, but just as much ice was formed at the lower as at the higher temperature. ST. JOHN (25) records that in the thick portion of egg white, freezing was complete in one hour at -12.5°C . There seems to be no doubt that the technique used in the present investigations allowed the freezing equilibrium point to be attained.

A nickel-plated pint Thermos food jar, with a top opening 2 inches in diameter, was used as a calorimeter. Exactly 250 gm. of distilled water, warmed to about 3°C . above room temperature, were poured into the calorimeter. As soon as the water had come to a temperature equilibrium with the calorimeter walls, as indicated by the calorimeter thermometer, the determination proceeded. The calorimeter thermometer was graduated to 0.02°C ., and had a temperature range of $+17^{\circ}$ to $+32^{\circ}\text{C}$. All the other thermometers used in these determinations were calibrated against the calorimeter thermometer.

After the sample had come to equilibrium with the bath temperature, the brass freezing tube was removed from the freezing bath, quickly wiped dry, and the sample transferred to the water in the calorimeter. This was accomplished by removing the rubber stoppers from each end of the tube, and while holding one open end of the tube over the calorimeter, hitting the inclosed sample a sharp blow with a short wooden rod of slightly smaller diameter than the brass tube. No difficulty was encountered owing to sticking of the sample to the walls of the freezing tube. The entire operation of transferring the sample to the calorimeter was completed within a few seconds.

As soon as the sample was immersed in the water, the calorimeter was stoppered and allowed to stand for a short period (3 minutes). After this the entire contents were shaken by inverting and righting

the calorimeter three times in quick succession. The calorimeter thermometer was then inserted, being mounted in a stopper which fitted the mouth of the calorimeter snugly, and the temperature read as soon as the contents had come to equilibrium. After this the calorimeter was shaken again in the same manner and the final temperature redetermined as a check on the first temperature. This check reading never differed from the first reading by more than 0.05° , and usually an even closer check was obtained.

The calculations of the amount of bound water present are based on the following considerations. The loss of heat energy in terms of calories by the water in the calorimeter is represented by the quantity:

$$FNS_w(T - T_e),$$

where F represents the calorimeter factor, N the number of grams of water in the calorimeter, S_w the specific heat of water for the temperature range $(T - T_e)$, T the original temperature of the water, and T_e the equilibrium temperature.

The heat lost by the water in the calorimeter will be utilized in five different ways as follows: (1) to warm the dry matter in the sample from the original temperature of the sample to the equilibrium temperature; (2) to warm the ice in the sample from the original temperature of the sample to the melting point of the ice; (3) to warm the unfrozen (bound) water in the sample from the original temperature of the sample to the melting point of the ice; (4) to warm *all* the water in the sample from the melting point of the ice to the equilibrium temperature; and (5) to melt the ice.

Each of these quantities is expressed mathematically as follows:

- (1) $W_d S_d (T_e - T_s)$
- (2) $W_i S_i (T_m - T_s)$
- (3) $W_b S_b (T_m - T_s)$
- (4) $W_w S_w (T_e - T_m)$
- (5) QW_i

Where:

W_d = weight of dry matter in the sample;

W_b = weight of bound (unfrozen) water in the sample;

$W_i = W_f$ = weight of ice (free water) in the sample;

W_w = weight of all water in the sample;

S_d = mean specific heat of dry matter for the temperature range indicated;

S_i = mean specific heat of ice for the temperature range indicated;

S_b = mean specific heat of bound water for the temperature range indicated;

S_w = mean specific heat of water for temperature range indicated;

T_s = temperature of sample;

T_m = melting point of ice in the tissue = freezing point of water in the tissue;

T_e = equilibrium temperature;

Q = heat of fusion of ice at T_m .

It is evident that the sum of these five quantities is equal to the quantity:

$$FNS_w (T - T_e).$$

Equating, and solving for W_f , remembering that $W_b = W_w - W_f$, the following equation is derived:

$$W_f = \frac{FNS_w(T - T_e) - [W_d S_d(T_e - T_s) + W_w S_w(T_e - T_s)]}{Q - [(S_b - S_i)(T_m - T_s)]}.$$

The values for T_s and T_m , being below zero, should be substituted in the preceding equation with a negative sign. By subtracting the amount of free water as calculated by the equation from the total water, the quantity of bound water present is determined.

The arguments followed in deriving the preceding equation follow in the main those found in the papers of THOENES (29) and ROBINSON (23). Their equations are also given by GORTNER (8). In the papers of ST. JOHN (25) and SAYRE (26), both of which appeared after the experimental part of this work was completed, the same general line of reasoning was followed. The final equation as here presented takes a slightly different, and in some respects it is believed, an improved form from that given by any of the aforementioned workers.

A correction factor for the calorimeter is necessary because a small

portion of heat lost by the water goes into the warming of the walls of the calorimeter and the thermometer instead of into the plant tissue. This factor was determined by introducing into the calorimeter masses of ice of known weight and of as nearly the same volume as possible as a 20-gm. sample of the pine leaves. Exactly the same procedure was followed as when determinations were made with the pine leaves. Since the number of calories required to change the temperature of ice and water and to melt the ice is known, a correction factor could readily be calculated from the values obtained for the change in temperature of the water. The factor for the system used in this work was found to be 1.06, this being the average value obtained from a number of closely agreeing determinations.

The specific heat of the dry matter of the pine needles was also determined in the calorimeter, following exactly the procedure previously outlined, except that benzene was substituted as the calorimeter liquid instead of water. Because of the high specific heat of water, it was found that the temperature changes when dry plant material was used were so small that the chances for errors were greatly enhanced. The use of a liquid of lower specific heat increased the probable accuracy of the determinations. The specific heats for benzene as given in the International Critical Tables (31) were used in these calculations. Obviously the calculation is simplified when a dry material is used, as the heat lost by calorimeter liquid goes only to warm up the dry material. The specific heat of the dry matter of pine needles was found by this method to be 0.30 calories.

The values used for the specific heat of water above zero are those given in the tables of the Handbook of Physics and Chemistry (12). The values used for the specific heat of water below zero were estimated by extrapolating the curve for specific heats of water as given in these same tables for values from -6° to -20° C. The values for the specific heat of ice and for the heat of fusion of ice are those given in the tables of DICKINSON and OSBORNE (5). SAYRE (26) gives a convenient summary table of the specific heats of ice and water, based on the same sources as used in this paper. The specific heat for bound water has been assumed to be the same as that of free water at the same temperature. The freezing (melting) temperature of the free water in the pine needles was assumed to be equal to the

freezing-point depression of the expressed sap. That this is probably not a strictly correct assumption is indicated by the work of CARRICK (3), who showed that the actual freezing temperature of the tissues of entire apple fruits is slightly lower than the freezing point of the sap expressed from the same organs. The same is presumably true for other tissues. The error involved is small, however, seldom exceeding 1°C. , and may be disregarded without any appreciable effect on the results.

Results and discussion

The results of the pressure dehydration experiments are presented in table I, in terms of the percentage hydration of the dry matter of the ground pine leaves when in equilibrium with each of the series of pressures used. The values for each of the eight individual determinations as well as the mean for each series are tabulated. The initial percentage hydration is much higher in the August series, owing of course to the greater water content of the tissues at that season. It is also possible to express a significantly larger amount of water from the tissues in the summer than in the winter. This, however, as is evident from the other figures in this table, is also due principally to the greater water content of the tissues at this season.

At all of the pressures used, the mean hydration percentages are slightly higher in the January series as compared with the August series. Comparison of these means was made by FISHER's method (7) of testing the significance of the difference of two means. The odds were only slightly in favor of any of these differences being significant; hence this method fails to demonstrate any unquestionable difference in the water-holding capacity of the leaf tissues in the winter as compared with the summer.

In a previous investigation (17), the writer found that pine leaves frozen at -20°C. in December showed a markedly greater resistance to dehydration under pressure than other samples of the same crop of leaves similarly treated the preceding August or the following June. This result, however, must be regarded as primarily due to the fact that freezing of the leaves in the summer resulted in death of most or all of the cells present, while in December little or no injury resulted. Such a determination is a measure of whether or not the

leaves have been injured by the temperatures to which they have been exposed more nearly than anything else. Similar, but much smaller differences were obtained with intact unfrozen leaves when they were subjected to pressure on the same dates. This seemed to indicate at that time an increased imbibitional pressure of the leaves in

TABLE I
PERCENTAGE HYDRATIONS OF GROUND LEAF SAMPLES

SAMPLE	PERCENT- AGE WATER CONTENT	SAP EX- RESSED PER 100 GM. TIS- SUE	PERCENTAGE HYDRATIONS OF DRY SUBSTANCE AT INDICATED PRESSURES (POUNDS PER SQUARE INCH)					
			0	375	750	1500	2000	3000
August 25, 1931								
1.....	63.4	46.2	174.0	68.0	61.8	54.5	51.1	47.7
2.....	63.1	41.9	170.0	73.4	67.7	63.3	59.4	56.9
3.....	62.5	41.2	166.7	73.7	67.5	62.5	60.0	56.9
4.....	62.8	42.3	170.0	75.8	66.0	60.9	57.1	55.3
5.....	63.2	43.6	174.0	70.6	65.9	59.7	56.5	54.4
6.....	62.5	44.2	166.7	63.8	57.1	52.9	50.9	48.6
7.....	60.1	42.1	150.0	59.9	55.5	49.8	47.4	44.9
8.....	60.1	38.9	150.0	67.8	61.3	59.5	55.3	53.0
Mean..	62.2	42.6	165.1	69.1	62.8	57.8	54.7	52.2
January 15, 1932								
1.....	58.3	34.4	141.0	71.0	67.4	63.3	58.9	58.0
2.....	57.5	30.8	135.2	75.7	71.1	66.4	64.2	63.4
3.....	57.2	33.7	132.5	68.5	64.0	58.3	56.3	53.8
4.....	57.7	32.2	138.1	75.3	69.8	66.4	64.3	61.5
5.....	57.4	37.1	135.2	59.2	54.0	52.6	50.6	49.0
6.....	56.6	32.2	129.9	70.6	65.3	60.9	57.8	56.1
7.....	57.1	33.1	132.5	68.2	64.2	58.2	56.7	55.2
8.....	56.8	33.8	129.9	64.8	60.0	56.7	54.1	52.5
Mean..	57.3	33.4	134.3	69.2	64.4	60.4	57.8	56.2

the winter as compared with the summer, but it now appears doubtful whether these results were actually of significance.

In contrast with the previous investigations in which intact leaves were used, the needles as used in this work were first coarsely ground before they were subjected to pressure. This was done to insure a more homogeneous packing of the press chamber and consequently a more equable distribution of pressure throughout the sample un-

dergoing pressure. If any significant difference in the imbibitional capacity of the cell colloids were present at different seasons of the year, it seemed probable that this technique would come closer to giving reliable indications of it than any other. As the previously presented data show, no indication of any appreciable difference has been found.

NEWTON (20), however, who was the first to use such a method as a measure or indication of cold resistance, was able to show rather marked differences in the hydration which would be maintained by the leaves of hardened wheat plants when placed under pressure as compared with the leaves of unhardened plants. The hardened leaves always maintained a higher hydration than the unhardened leaves at the same pressure, in spite of the fact that the initial water content of the unhardened leaves was always greater. These results were obtained with intact leaves, untreated in any way.

MARTIN (14), working subsequently, was able in general to confirm NEWTON's results with wheat plants. The volume of press juice expressed usually showed an inverse correlation with the relative hardness of the variety the leaves of which were subjected to pressure. Such results were obtained with both frozen and unfrozen leaves, although a number of irregularities occurred. For reasons mentioned in a preceding paragraph, it appears to the writer that the results of pressure-dehydration determinations made with unfrozen leaves are much more likely to be significant than those made with frozen leaves.

STEINMETZ (28) also employed this method on alfalfa roots, but could find no correlation between the volume of juice expressed and hardness. Since he first froze his samples at a low temperature, however, thus probably killing all or most of the cells, such a result is not surprising.

The results of the calorimetric determinations on the 1931 leaves of the pitch pine in August, 1931, and January, 1932 are presented in table II. Eight separate samples were determined from collections on each of these dates, each sample being from a different tree. Data are given for the total water, dry matter, free water, free water per gram of dry matter, bound water, and bound water per gram of dry matter for each, as well as the mean for each set of eight figures.

The mean value for each of the August determinations was compared with the corresponding mean as determined in January, by FISHER'S method of testing the significance of the difference between two means. The odds were overwhelming that there was a signifi-

TABLE II
RESULTS OF CALORIMETRIC DETERMINATIONS OF BOUND
AND FREE WATER IN PINE NEEDLES

SAMPLE (20 GM.)	WATER (GM.)	DRY MATTER (GM.)	FREE WATER (GM.)	FREE WATER PER GM. DRY MATTER (MG.)	BOUND WATER (GM.)	BOUND WA- TER PER GM. DRY MATTER (MG.)
August 25, 1931						
1.....	12.67	7.33	9.33	1272	3.34	456
2.....	12.63	7.37	9.07	1231	3.56	483
3.....	12.50	7.50	9.14	1219	3.36	448
4.....	12.54	7.46	9.47	1269	3.07	411
5.....	12.64	7.36	9.23	1254	3.41	463
6.....	12.51	7.49	9.41	1256	3.10	413
7.....	12.08	7.92	9.03	1140	3.05	385
8.....	12.05	7.95	8.91	1120	3.14	395
Mean.....	12.45	7.55	9.20	1220	3.26	432
January 15, 1932						
1.....	11.66	8.34	8.60	1031	3.06	367
2.....	11.49	8.51	7.82	919	3.67	431
3.....	11.44	8.56	8.13	950	3.31	387
4.....	11.54	8.46	8.02	948	3.52	411
5.....	11.48	8.52	8.51	999	2.97	349
6.....	11.31	8.69	8.12	934	3.19	363
7.....	11.42	8.58	8.40	979	3.02	353
8.....	11.38	8.62	8.21	952	3.17	368
Mean.....	11.46	8.54	8.23	964	3.24	379

cant difference between every pair of means, except the two for the total amount of bound water present.

The total water content of the samples is less in winter than in summer, the dry matter content showing a reciprocal relation of course. This decrease in water content in passing from the summer to the winter condition has been observed in this species during a number of previous years. This means also, as shown in table I, that

the total hydration of the tissues is less in the winter than in the summer.

The quantity of free water present is larger in the summer than in the winter. This difference is further emphasized when the calculations are made on the basis of a unit of dry matter, which reduces the values to a more nearly comparable basis in terms of the actual substance involved. The higher free-water content in the summer appears to bear a close correlation with the higher total hydration of the leaf tissues at that season. That is, with decrease in the total hydration of the leaf tissues there is a decrease in the amount of free water present, and vice versa.

The total quantity of bound water present in the winter is not significantly different from the quantity present in the summer. Calculations based on a unit of dry matter, however, indicate a significantly greater amount of bound water in the summer as compared with the winter. Data regarding the total amount or percentage of bound water in any colloidal system in themselves reveal very little about the physico-chemical properties of that system; only when such data are referred to the dry matter content do they become intelligible. Although the total amount of ice formed in equal weights of leaves is greater in the summer than in the winter, the residual hydration of the dry matter after freezing of the leaf tissues is greater in the summer than in the winter. Decrease in the total hydration of the system appears to result in a decrease in the amount of bound water as well as of free water.

During the year 1930-1931, a similar series of bound-water determinations was made on the 1930 leaves of the pitch pine. These results are not reported in the present paper because the technique employed in obtaining them was not quite up to the standards adopted for the 1931-1932 series. The results were concordant with those under discussion, however, in that they also indicated a higher proportion of bound water per gram of dry matter in the summer than in the winter.

As-yet unpublished work from this laboratory by Miss CHRYSLER on the amounts of bound and free water in a plant colloid (the stipe of the Elk kelp, *Pelagophycus porra*) at different degrees of hydration has resulted in substantially similar conclusions. These results

are also supported by data on various colloidal systems by NEWTON and MARTIN (22), BRIGGS (2), and JONES and GORTNER (13). In general, therefore, it appears that the amounts of bound and free water in a colloidal system are positively correlated with the total degree of hydration of the system. This is, in fact, what would be expected if the bound and free water in a system are considered to be in equilibrium. Plant tissues, the present work shows, are no exception to this principle, and for the purpose of such a conception may be regarded as representing exceedingly complex, hydrated colloidal systems.

These results conflict with those of several preceding investigators who have used a measure of the unfreezable water in plant tissues as indication of the amount of bound water present, and whose work indicates a correlation between the amount of bound water present and cold resistance.

ROSA (24) appears to have been the first to present experimental data which favored the view that increasing hardness is correlated with an increase in the bound water content of the tissues. He used cabbage plants in most of his experiments, employing the dilatometer method as developed by MCCOOL and MILLAR (16) for determining the amount of water which would freeze. The percentage of the total water freezing at temperatures down to -6°C . was found to be less in hardened cabbage plants at every temperature than in unhardened plants. Conversely, the total amount of water remaining unfrozen was greater at every temperature in the hardened as compared with the unhardened plants. Furthermore, it was found that the percentage of the total water in cabbage plants which could be frozen at -5°C . decreased progressively in the period of 20 days during which the plants were being hardened in a cold frame.

At first glance it would appear that these results are completely at variance with those reported in the present paper. Further consideration shows, however, that the discrepancies are not so great as they appear. ROSA's results are presented in terms of grams of water remaining unfrozen in 100 gm. of tissue. Since during the hardening of the cabbage plants there was always an increase in dry weight, the calculation of his results in terms of the grams of water remaining unfrozen per gram of dry weight markedly reduces the increase in

bound water found in hardened leaves as compared with unhardened ones. As previously pointed out, this is probably the most significant terminology in which to express the amounts of bound water present. Furthermore, the lowest temperatures used by ROSA were -5° and -6° C., instead of -20° C. as used in the present work. It seems probable that had such a lower temperature been used, the differences found in the amounts of bound as compared with free water might have been still further reduced. This seems especially true, since ROSA allowed only a very short time for equilibrium to become established in his determinations. JONES and GORTNER (13) have shown in their experiments that a relatively long period is required for freezing in organic colloids to become complete at a temperature of -11.1° C., while equilibrium is usually attained much more rapidly at a temperature of -20° C. Furthermore, although in some colloidal systems (13) as much water freezes at -6° C. as at -20° C., if sufficient time is allowed for the attainment of equilibrium, this does not prove that the same is true for plant tissues in which the liquid medium is not water, but a solution sometimes of considerable concentration.

Finally there is the possibility that this increase in unfreezable water is due, in part at least, simply to the increase in soluble carbohydrates, which, although not great, is shown to occur during the hardening of cabbage plants by ROSA's own analyses. MAXIMOV (15), CHANDLER (4), and ÅKERMANN (1) especially have championed the conception that the higher the concentration of soluble carbohydrates in a plant cell the smaller the proportion of water which will freeze at any given temperature below 0° C.

In a molar solution of sucrose, for example, it is known that about 50 per cent of the water will freeze at about -4.336° C. (the freezing point depression of a 2M sucrose solution according to BRIGGS, 2), while only traces of ice will form in a 2M solution at the same temperature. The possible relation of this to cold resistance is elaborated in the papers of the three investigators just mentioned. Other solutes would be equally effective, providing only that their eutectic points are below that of the freezing temperature. Although this is a well known physico-chemical fact, and there seems no reason to believe that cell sap solutions would not behave in the same way, the direct

evidence from plants on this point is still fragmentary. Nevertheless this remains an important possibility, and it seems likely that some of the increase in bound water which occurs in hardened plants is simply due to the effects of the increased soluble carbohydrate content of the cell sap, which is an almost invariable accompaniment of an increase in hardness.

ROBINSON (23) has made an investigation of the relation of bound water to the hardening process in insects. The calorimeter method was used, the exact technique being similar to that followed in the present investigation. When the larvae of the Prometheus moth (*Callosamia promethea*) were gradually cooled in refrigerating cabinets from 18° C. (temperature of the cabinets) until the internal temperature of the insects was reduced to -6.5° C. at a cabinet temperature of -14° C., a marked increase in the amount of bound water present at the expense of the free water occurred. Maximum values for bound water were attained in about two weeks' time. When similar experiments were conducted with non-hardy species, either there was a very slight increase in bound water or else an actual decrease.

Attention should be called to the fact that the term "hardening" as used by ROBINSON with reference to insect larvae does not have the same meaning as the definition adopted for the term in this paper. His experiments were conducted at temperatures above the actual freezing points of the larvae, so that ice did not form in the tissues at any time, except during the calorimetric determinations. In the pitch pine and other species to which this paper has special reference ice often forms in the tissues. There are, as is well known, hardy and non-hardy plants with respect to the range of low temperatures just above the point of actual ice formation in the tissues, but it is not hardness of this type which is at present under consideration. There are also species of insects the larvae of which will survive the formation of ice in the tissues, but ROBINSON's work did not deal with such conditions.

These results do not agree with those reported for pitch pine in the present paper, and only further investigation will eliminate or explain the discrepancies. It is possible that bound water may play a more important rôle in the hardening of insect larvae than in the hardening of plants. ROBINSON believed that the increase in bound

water as observed in his experiments was due principally to an increased adsorption of water, presumably on colloidal surfaces, at the lower temperatures. It is possible that a gradual cooling of living tissues over a period of a week or more, as in ROBINSON's experiments, might cause a shift in the bound-free water equilibrium toward the bound water side. The natural conditions to which the pitch pine leaves had been exposed prior to the winter determinations recorded in this paper were not identical with the conditions employed by ROBINSON. The pine leaves, however, had just been exposed to a period of more than a week of cool temperatures, and were unquestionably in the hardened condition, although the temperatures to which they were exposed were intermittent and not as low as those in ROBINSON's experiments.

There is another important difference between the conditions in ROBINSON's experiments and those reported in the present paper. The insect larvae with which he worked showed very little variation in total water content, while the hardened pitch pine leaves had a distinctly lower water content than the unhardened leaves. As pointed out previously, the amount of bound water in the leaves of these species appears to correlate directly with the total hydration of the leaves. Any effect which lowering of the temperature might have in increasing the amount of bound water, or adsorbed water, may be obscured in the pitch pine leaves by the decreasing effect of a lower total hydration of the leaves. Experiments are projected to test the exact effects of controlled changes in temperature upon the amount of bound water in pitch pine leaves under such conditions that little or no change in the total hydration of the tissues occurs.

Another difficulty arises in attempts to compare bound-water values obtained for plant tissues with those obtained for animal tissues, owing to the much more prominent rôle played by the cell wall in plants. NOVIKOV, as cited by MAXIMOV (15), found that wheat leaves which had been killed with chloroform gave the same values for bound water determinations as living leaves. This and other considerations suggest that the great bulk of the bound water in plant tissues may be in the cell walls. A very large proportion of the dry matter of leaf tissues is to be found in the cell walls; much of this substance is hydrophilic; and theoretically it would be expected to con-

tain considerable quantities of water in that physical condition usually spoken of as bound water. This conception would relegate the protoplasm, and cell contents generally, to a relatively minor part in the binding of water in plant tissues, and helps clarify the similarity in behavior between non-living colloidal systems and living tissues with respect to the effect of the total hydration of the system on the amounts of bound and free water present. The smaller the quantities of solutes present, with their colligative effects on the amount of water freezing in a system, the closer the similarity which might be expected between living and non-living systems.

It is evident that the results of this investigation offer no support for the hypothesis that the binding of water by colloids is an important factor in the cold resistance of the pitch pine. Presumably the same is true for the leaves of other evergreens. This does not eliminate the possibility, of course, that colloids play a rôle of importance in the phenomenon of cold resistance. They may operate through some other mechanism than that of preventing the freezing of water. It is conceivable that there may be increases in the actual quantities of material in the colloidal condition, or even sol to gel transformation, without any marked effect on the amount of bound water present. Certain types of colloids might operate to stabilize the protoplasm against the desiccating effects of freezing. Stabilization of inorganic sols in this way can readily be demonstrated. Another possibility is that changes in the colloidal organization of the cell contents might work in such a way that the size of the ice crystals or mode of crystallization might be affected.

In the last analysis it seems clear that no physico-chemical condition has been discovered which is an invariable accompaniment of the state of cold resistance in plant cells. Some conditions, such as an increased sugar content of the cells, frequently correlate with the ability of plant cells to survive exposure to low temperatures; but there are always important exceptions. None of these correlating factors is definitely known to be causatively related to cold resistance. It seems probable that the ultimate basis for this phenomenon lies in the protoplasm itself, and that attempts to resolve the problem by gross physiological measurements are doomed to be unsuccessful. Except in tissues of very low water content, in which the

protoplasm is initially at a low hydration percentage, exposure of plant cells to low temperatures apparently always results in a more or less severe desiccation of the cells owing to the conversion of a large portion of the water present into ice. The severity of this desiccation depends to some extent upon the temperature to which the cells are subjected, the duration of the exposure, and, for any given species, upon the exact physiological conditions which prevail in the cells at the time of freezing. It seems probable, therefore, that the ultimate consideration in hardiness and cold-resistance phenomena is whether or not the protoplasm itself can endure this desiccation. Protoplasms which can endure desiccation of this type will survive low temperatures without destruction; those which cannot will be destroyed or severely injured. In many species the same protoplasmic units may at times be able to survive the condition, while at other times they cannot. In the last analysis, the basis of cold resistance is almost certainly to be found in the physico-chemical organization of the protoplasm itself. Until more precise methods of investigating protoplasm from this standpoint are devised, it appears that further progress in the problem will be very slow. MAXIMOV (15) considers the basic factor in drought resistance to be the ability of the protoplasm to endure periods of desiccation, due to loss of water, without injury. There appear to be some marked similarities in the physiological mechanisms which lie at the basis of these two properties of plants. This analogy, although enticing, must not be pushed too far, as curiously enough there are many plants the protoplasm of which can endure true desiccation as engendered by drought conditions, but cannot endure the physiological desiccation caused by ice-crystal formation in the tissues.

Summary

1. Seasonal variations in the cold resistance of the leaves of evergreen species are discussed in terms of the present knowledge of such phenomena. Evergreen leaves, in general, are not cold-resistant during the warmer months of the year. Time and temperature factors involved in the hardening of the leaves of such species are analyzed in terms of known facts. The work of several investigators indicates that the threshold temperature for hardening seems to be about

+6° C. A few hours' exposure to this or a lower temperature per day suffices to keep the leaves in the hardened condition. During warm spells in the winter the leaves of some species of plants seem to lose their hardiness very quickly; in other species the physiological changes causing loss of hardiness appear to occur much more slowly.

2. In this investigation attention was directed to further studies bearing on the hypothesis that an increase in bound water is an important factor in the cold resistance of the leaves of evergreen species. A pressure dehydration method of distinguishing between bound and free water in plant tissues is outlined. A modified form of the calorimetric procedure of determining the amount of free water in a system, suitable for use with plant tissues, is described. An improved form of the equation for calculating the amount of free water from the calorimetric data is also derived.

3. The leaves of the pitch pine (*Pinus rigida* Mill.) were used as the material for investigation. The total amount of water in the leaves of this species was about 5 per cent less in the winter as compared with the summer. Larger amounts of sap can be expressed from these leaves in the summer than in the winter, but this difference represents almost entirely a correlation with the difference in total water content. No evidence could be obtained by the pressure dehydration method of any significant increase in the amount of bound water in the pine leaf tissues in the winter as compared with the summer. The calorimetric determinations showed greater amounts of both bound and free water per gram of dry matter in the leaves of this species in the summer as compared with the winter. The differences between the summer and winter values were statistically significant. Apparently, as has been found previously in non-living colloidal systems, there is a positive correlation between the total hydration of the tissues and the amounts of both bound and free water per gram of dry matter. No evidence was obtained, therefore, that increase in bound water plays any rôle in the cold resistance of this species.

4. The results of other investigations are critically analyzed in relation to the results of this investigation. It is suggested that the basis for cold resistance lies in some as yet not understood physico-chemical properties of the protoplasm, which probably cannot be

discovered by the gross measurements which are generally employed at present.

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CYTOLOGY OF ANTENNARIA¹

II. PARTHENOGENETIC SPECIES

G. LEDYARD STEBBINS, JR.

(WITH PLATES II, III AND THIRTEEN FIGURES)

The purpose of this investigation, as well as the history of the subject and the material and methods, was outlined in a previous paper (STEBBINS 38).

Observations

DEVELOPMENT IN STAMINATE FLOWERS

In the seven species of *Antennaria* here studied, which reproduce entirely (or almost entirely) parthenogenetically, the staminate plants are rare or unknown. In only three could material for a study of the microsporogenesis be obtained.

A. fallax Greene, staminate material from a dry, scantily wooded knoll near Canton Junction.—The plant is larger in all its parts than *A. plantaginifolia*, its nearest relative, and the cells are all of considerably greater size. At diakinesis both paired bivalent chromosomes and unpaired univalents are present. At the heterotypic metaphase the bivalent chromosomes are arranged at the equatorial plate, while from two to twelve univalents are scattered over the spindle (fig. 14). Enough counts were made on favorable metaphase cell plates to fix the haploid number at forty-two. Figure 15 shows a metaphase cell plate at which forty pairs of bivalent and four univalent chromosomes can be counted. The univalents can be recognized from their lesser depth of focus, and they are frequently at a different plane of focus from the cell plate.

The univalents lag near the center of the spindle, or are scattered in the cytoplasm during the heterotypic anaphase, and generally form one or two extra nuclei during interkinesis. The homoeotypic division is for the most part regular, and figure 17 shows a homoeotypic metaphase in which 41 chromosomes may be counted in one

¹ Contribution from the Laboratory of Plant Morphology, Harvard University.

cell plate, one chromosome having been ejected into the cytoplasm. There are often extra nuclei at the homoeotypic telophase, and about 60 per cent of the "tetrads" are of the polysporic type. The pollen is about 50 per cent perfect.

Cytomixis was frequently observed in *A. fallax*, mostly during the prophase. Figure 16, however, shows a heterotypic anaphase where cytoplasmic connections with a neighboring cell have persisted. Figure 18 shows a homoeotypic telophase in which two of the nuclei of one cell are stretched across and invade the cytoplasm of a neighboring cell. Both cells show signs of shrinkage and degeneration, but neighboring cells in the same anther locus were perfectly normal in appearance.

The staminate plants of *A. fallax* studied are of the "aberrant male" type described by JUEL (21) in *A. alpina*. The flower heads are higher than those of pure staminate flowers, the pappus hairs have only slightly dilated tips, the style is longer than in typical staminate flowers, while the stigma has two short branches instead of a single capitate tip. Achenes develop in 2 to 25 per cent of the flowers in each head, the average being about 10 per cent. In these achenes, a megaspore mother cell may be seen, first in the spireme stage, and later in diakinesis. At the heterotypic metaphase, there are bivalents at the equatorial plate, while a large number of univalents are scattered about the spindle (fig. 36). The heterotypic division is almost regular; and, although no chromosome counts could be obtained at any stage, owing to scarcity of material, reduction appears to have taken place (fig. 37). However, fusion may possibly take place, after the homoeotypic division, between the two daughter cells of the spindle at the chalazal end of the nucellus. The only megaspore group observed contained only three large nuclei, that at the chalazal end being distinctly larger than the other two (fig. 38). The development of the embryo sac, owing to scarcity of material, could not be perfectly studied.

A. parlinii Fernald, staminate material from dry field, Ballston, Virginia.—The flowers are of the aberrant male type, although the proportion of developed achenes is smaller than in *A. fallax*. No stages of meiosis in the achenes were found.

In the pollen mother cells there are univalents, bivalents, and pos-

sibly trivalents and quadrivalents at diakinesis. At the heterotypic metaphase from ten to sixteen univalent and loosely paired chromosomes are scattered over the spindle (fig. 19). In polar view, the chromosomes are clumped together in groups so that counting is difficult. Counts ranged from 39 to 46 as the haploid number; but in the most favorable cells a haploid number of 42 was more or less clearly seen. Figure 20 shows such a cell, with 37 bivalents and 10 univalents. Here most of the bivalent pairs are grouped into quadrivalent and sexivalent associations. There is more lagging during the heterotypic division than in *A. fallax*, and 90 per cent of the "tetrads" are polysporic (fig. 22).

Rarely the heterotypic division fails of completion, and a "restitution nucleus" results. This nucleus is most often dumb-bell shaped, but may be irregular in form, as in figure 21. This restitution nucleus evidently divides with a division of the homoeotypic order, resulting in a dyad of microsporocytes, each of which has the diploid number of chromosomes (fig. 23).

A. canadensis Greene.—The following staminate plants were studied: no. 540, grassy hillside, Lexington. Leaves not so bright green as in typical *A. canadensis*; stolons assurgent; cauline leaves with scarious appendages, although these are not so well developed as in typical *A. canadensis*. Although this plant shows some transitions toward *A. neodioica*, all the pistillate plants found in the immediate vicinity of its locality were typical *A. canadensis*. Flowers were of the aberrant male type. No. 757; Canton Junction. The plants correspond in every respect to typical *A. canadensis*. Flowers were of the pure male type.

In no. 540, the diakinesis stage was not often found. At the heterotypic metaphase there are generally a few paired bivalents and a large number of univalents. At the heterotypic anaphase the bivalent pairs split and move toward the poles, so that the chromosomes are scattered irregularly over the spindle. In favorable spindles, at this stage, approximate counts can be made, although the large number precludes absolute accuracy. Figure 24 shows 83 chromosomes, which is about the diploid number for *A. canadensis*. This stage is found very frequently, and apparently lasts for a long time. The spindle becomes gradually narrower and the chromosomes more

clumped together, while the spindle may become bent, as in figure 25. The subsequent development occurs in one of two ways:

1. The chromosomes may all, or nearly all, reach the poles, and be inclosed in the daughter nuclei at the heterotypic telophase. Here the homoeotypic division proceeds regularly, and results in a tetrad or polysporic group. Figure 31 shows a homoeotypic metaphase cell plate, where 40-42 chromosomes, the haploid number, can be counted.

2. The heterotypic telophase may set in before the chromosomes have reached the poles, and a restitution nucleus may thus be formed (fig. 26). The chromosomes then go through the interkinesis stage, apparently losing their identity, while a nucleolus frequently appears. Later they are seen in the prophase of the homoeotypic division, appearing longer and more slender than in the heterotypic division, and many show clear evidence of splitting (fig. 27).

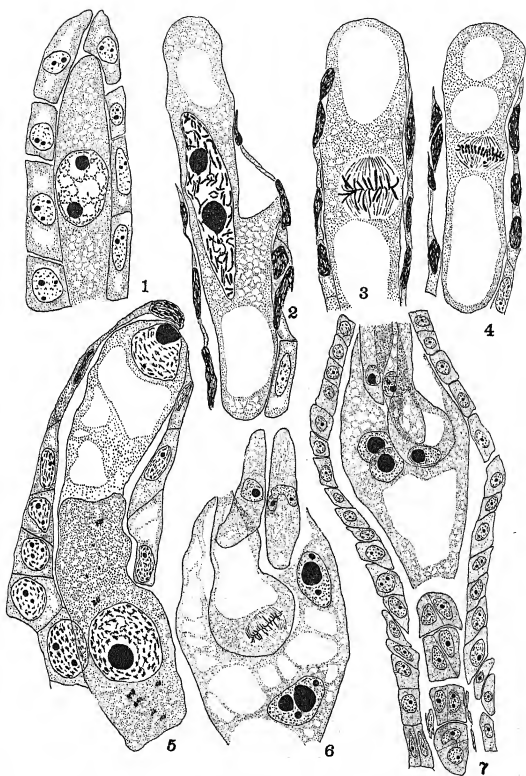
A homoeotypic, equational division then takes place, at which there is only one spindle (fig. 28). Figure 29 shows the polar view of such a spindle, at which about 86 chromosomes can be counted, clearly the diploid number. The division proceeds for the most part regularly, although figure 30 shows a telophase at which lagging chromosomes have formed a bridge between the daughter nuclei. The result is a dyad, although one or more microcytes may be present.

The percentage of occurrence of these types of development is indicated by the relative abundance of the various types of microspore groups, which is: dyads 27, tetrads 34, polysporic "tetrads" 39 per cent. Many of the apparently normal tetrads must later degenerate, as practically all of the mature pollen grains are evacuate or otherwise abnormal in appearance.

In no. 757 the development is much the same, and the chromosome number, although it could not be counted accurately, is about the same. The percentage of the various types of microspore groups is: dyads 37, tetrads 8, polysporic groups 55 per cent.

DEVELOPMENT IN PISTILLATE FLOWERS

In all of the seven parthenogenetic species there are two types of development in the pistillate flowers, a general description of which will, with slight deviations, apply to all.



FIGS. 1-7.—Fig. 1, *A. fallax*, embryo sac mother cell. Fig. 2, *A. petaloidea*, prophase, first embryo sac division. Fig. 3, *A. canadensis*, normal metaphase of same. Fig. 4, *A. canadensis*, no. 532, abnormal metaphase of same. Fig. 5, *A. parlinii*, archesporial cell replaced by integumentary one. Fig. 6, *A. petaloidea*, first division of egg nucleus. Fig. 7, mature embryo sac. Figs. 1-5, $\times 1200$; figs. 6, 7, $\times 600$.

The first is the type described by JUEL (21) for *A. alpina*, and designated by ROSENBERG (35) as the *Antennaria* type. Here the young megaspore mother cell does not show a definite spireme stage. At the age in which the spireme is seen in the megaspore mother cells of *A. plantaginifolia*, and of the "aberrant males" of *A. fallax*, there are frequently a few thin threads formed, but these soon break up again into chromatin granules (fig. 1), and soon these granules are scattered at random throughout the nucleus. At all stages the nucleus stains weakly, except for the nucleoli. As the young embryo sac increases in length, the nucleus increases in size but does not divide. The formation of a tetrad of megaspores is entirely omitted. As the nucellar cells start to degenerate, the nucleus moves out to the micropylar end of the young embryo sac, and a vacuole forms at the chalazal end. When most of the nucellar cells have degenerated and the embryo sac has pushed out beyond them, this mother nucleus divides for the first time. This division is carried through very quickly, and its stages are difficult to find. At the later prophase the nucleus is often very much elongated, and there are a large number of long, slender, unpaired chromosomes (fig. 2). This resembles closely the similar stage in *A. alpina* and in *Taraxacum albidum* (OSAWA 27). An interesting feature in figure 2 is the very clear budding of the lower nucleolus, a phenomenon seen only in this instance. At the metaphase the chromosomes are all of the long, slender, somatic type, and are arranged as in an ordinary somatic division (fig. 3). The daughter cells resulting from this division migrate to either end of the embryo sac, and there undergo the two somatic divisions which result in the young 8-celled embryo sac. It was impossible to obtain chromosome counts at any of these divisions, owing to the large number and the interlocking of the very long and slender chromosomes; but in *A. fallax*, *A. petaloidea*, and *A. canadensis* the number was definitely determined as greater than the haploid number for the species.

The embryo sac grows into the usual flask shape, although the polar nuclei do not fuse, but lie next to each other against the egg cell until just before the endosperm begins to develop (fig. 7). The number of antipodals is only eight to ten, less than that found in the mature embryo sac of the non-parthenogenetic species (STEBBINS 38).

In figure 7, the two antipodal nuclei at the chalazal end of the group are connected through the cell membrane separating them by a thread of nuclear material. This was probably caused by the accidental lagging of one of the chromosomes during the late anaphase of the division to form these nuclei. Similar threads connecting antipodal nuclei, where, however, no cell membrane separated them, were found in *Artemisia maritima* by WEINEDEL-LIEBAU (41), and interpreted as evidence of amitosis. It is doubtful, however, that such has taken place here.

The first division of the egg cell takes place when the endosperm is 2- or 4-nucleate (fig. 6). The resulting daughter nuclei are separated by a horizontal wall, and that nearest the micropyle fails to divide further. The next few divisions result in an embryo with a 2-celled suspensor. In the meantime the endosperm has divided much more rapidly, and contains a large number of nuclei when the embryo is only 4-nucleate. At no time was any sign of a pollen tube seen, either in the micropyle or at the base of the embryo sac, although abundant material of these stages in *A. petaloidea*, *A. neodioica*, *A. canadensis*, and *A. parlinii* was studied.

The second type occurs much less frequently, but some stages of it were found in each of the seven species. Here there is a definite spireme in the young megaspore mother cell (fig. 32), and the spireme contraction or synizesis is present (fig. 33). Diakinesis occurs at the same stage as in the non-parthenogenetic species, but both paired and unpaired chromosomes are present, while the nucleus is very large (fig. 8). At the heterotypic metaphase there are a few chromosomes at the equator (fig. 9), but the majority are scattered irregularly over the spindle. Since there are a number of paired bivalents present, however, this cannot be called a true semiheterotypic division.

The location of the dense layer of the perinuclear zone in figure 9 is interesting. It forms a large loop extending from the micropylar end of the spindle, and is entirely absent from the chalazal end. The inner edge of this loop probably corresponds with the position of the nuclear membrane during diakinesis. The spindle has apparently formed considerably nearer the chalazal end of the nucellus than the original position of the nucleus at diakinesis.

During the early anaphase (fig. 10) the spindle elongates, and reoccupies the micropylar end of the nucellus. Here, and in all the later stages, the major part of the perinuclear zone is at the chalazal end of the nuclear structures. The chromosomes gradually and irregularly move toward the poles, so that they are scattered over the whole length of the spindle. Non-disjunction is common, and paired chromosomes may be seen at both poles. This was the stage most frequently observed of any in the meiotic divisions, and apparently lasts for a considerable period of time. In figure 11 the chromosomes have migrated farther toward the poles, have lost their identity, and are merging together, while a number of them have been ejected into the cytoplasm. The spindle is thinner than ever, and is distinctly bent.

At the heterotypic telophase a large mass of chromatin at the center of the spindle is left out of the two daughter nuclei, and forms a third nucleus (fig. 42). Figure 12 shows a homoeotypic metaphase at which there are three spindles instead of the usual two. This division results in a hexad (fig. 13), although slight irregularities in it frequently cause the presence of small extra nuclei.

The further development of these hexads is problematical. That reduction has taken place is certain, from the distribution of the chromosomes during the heterotypic anaphase; and each cell of the hexad probably has fewer than the normal, haploid number of chromosomes. Figure 49 shows a pentad in which the chalazal cell is developing at the expense of the micropylar ones. This cell has a nucleus distinctly larger than the others, and may have resulted from the fusion of the two daughter nuclei formed by the chalazal spindle of the homoeotypic division. In this case, of course, it would have a number of chromosomes approximating the diploid number for the species.

Figure 50 shows a hexad in which the cell next to the micropylar cell, and that next to the chalazal one, are larger than the other four, and are apparently both starting to develop. Figure 51 shows a very abnormal 4-celled embryo sac, apparently resulting from the situation of figure 50. It is in two parts, the two nuclei at the micropylar end probably having been formed from one cell of a hexad, and the two at the chalazal end from another. The remains of two more of

the hexad cells are seen just below the chalazal end of this "embryo sac." It is doubtful whether such an embryo sac can develop further, and no other similar one was seen at any stage. All the mature embryo sacs are perfectly normal in appearance, and approximate counts in the later divisions showed that the diploid number is most likely present in all where such counts could be made. A few embryo sacs showed obvious signs of degeneration.

In *A. occidentalis*, bagging experiments of the type described in a previous paper (STEBBINS 38) showed 549 out of 1084 achenes, or 51 per cent, developing in the bagged inflorescences; while in the unbagged material 617 good achenes out of 1106, or 56 per cent, were counted. This difference may be due to a number of causes, such as the more favorable conditions for development in the unbagged heads. The possibility of the fertilization of the egg cells in a few achenes in the unbagged material is slight, as there were no staminate plants of *A. occidentalis* in the vicinity, and the pollen of the staminate plants of the other species had long been shed when the stigmas of *A. occidentalis* first opened. In the other species, in unbagged material, from 50 to 80 per cent of good achenes was found. From the frequency of the second type of development, which will be discussed later, it seems most likely that all of the achenes in which this abnormal reduction division takes place, as well as some in which there is no reduction division, degenerate.

The following material of the various species was studied:

A. occidentalis Greene, no. 536, field, Winchester, Mass.; no. 539, field, Lexington.—In this species the abnormal reduction division is more frequent than in any other, occurring in about 25 per cent of the achenes, 75 per cent developing according to the *A. alpina* type. The diploid chromosome number is about 75–80, although no certain counts could be made.

A. fallax Greene, no. 521, field, Lexington; no. 522, field, Monponsett, Halifax, Mass.—In no. 522, development was entirely of the *A. alpina* type, but in no. 521 various stages of the abnormal reduction division were found in one bud (figs. 32, 34, 35). The chromosome number could not be counted in the pistillate material, but from the staminate plants it is estimated at $2n=84$.

A. parlinii Fernald, no. 529, meadow, Orange, Mass.; no. 541, hill-

side, Lexington; no. 745, open woods, East Riverdale, Maryland; no. 747, field, Ballston, Virginia.—No. 747 differs from the other plants in its smaller, less abundant red glandular hairs, its broader leaves, and its less acuminate, more petaloid involucral bracts, corresponding therefore to var. *arnoglossa* (Greene) Fernald.

In nos. 529 and 541 development is entirely of the *A. alpina* type. In no. 541, the diploid chromosome number, estimated from one of the embryo sac divisions, is about 82. In no. 745, rare evidences of the abnormal reduction division were found, including a tetrad with seven nuclei (fig. 52). In no. 747 the development is mostly of the *A. alpina* type, but one clear case was found of the unreduced megaspore mother cell being replaced by a cell from either the chalazal end of the nucellus or the inner integument (fig. 5). Possible older stages were seen, in which the invading cell, with its dense cytoplasm filled with dark granules, had entirely filled the nucellus, although the remnant of the degenerated archesporial cell could be made out.

A. canadensis Greene, no. 269, dry field, Wilmington, Mass.; nos. 525 and 526, Halifax, Mass.; no. 532, Beverly; and no. 545, Tewksbury.—Development is almost entirely of the *A. alpina* type, the only evidence of a reduction division found being a single old tetrad in no. 526 (fig. 39). However, a single exception to the usual type of metaphase of the first embryo sac division was found in no. 532 (fig. 4). Here the chromosomes are much smaller than in the usual type of metaphase (fig. 3), and are comparatively shorter and thicker. They do not seem to be in pairs, and the division is probably equational; but there are, nevertheless, four chromosomes scattered over the spindle, apparently laggards. The shape of the chromosomes is much the same as in the homoeotypic division of meiosis, and their behavior is apparently the same. No abnormalities preceding this stage were seen, and no prophase of the first embryo sac division were found in no. 532. The metaphase, in appearance and distribution of the chromosomes, resembles the only one seen and illustrated by JUEL (21) in the first embryo sac division of *A. alpina*. Here he remarks on the presence of a few chromosomes not at the equator of the spindle, and suggests that they have been displaced by the knife. In the case of no. 532, however, the cell plate was not touched in sectioning.

A. petaloidea Fernald, no. 538, field, Arlington Heights; no. 527, Bryantville, Mass.—The latter plant corresponds to var. *noveboracensis* Fernald. In no. 538, very few of the younger stages were observed, and these all showed the *A. alpina* type of development. In no. 527, the abnormal reduction division occurs in about 20 per cent of the achenes (figs. 40–43). In the homoeotypic metaphase in figure 43, about 36 chromosomes can be counted on one spindle and about 40 on the other. No other chromosome counts could be obtained in *A. petaloidea*, so that the diploid chromosome number can be estimated at 75–80.

A. neodioica Greene, no. 268, clearing, Wilmington, Mass.; no. 523, field, Monponsett; no. 533, clearing, Beverly Farms, Mass.—In nos. 268 and 533, only the *A. alpina* type of development was found; but in no. 523 the abnormal reduction division occurs in about 10–15 per cent of the achenes (figs. 44–47). Such chromosome counts as could be made from these stages fixed the diploid number as near 52.

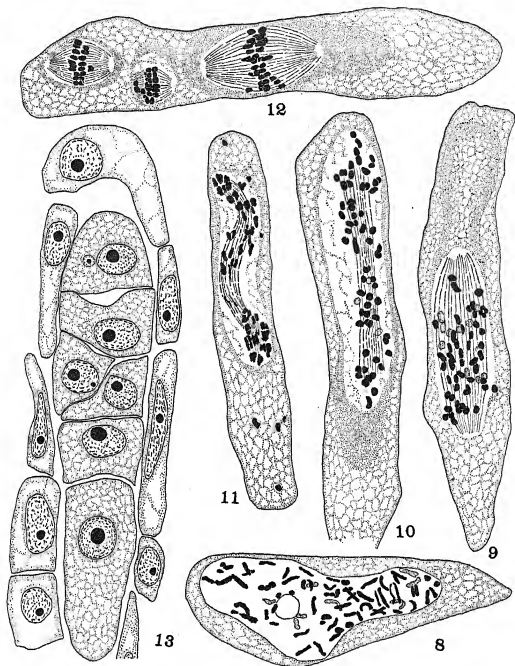
A. neodioica Greene var. *attenuata* Fernald, no. 266, open woods, Dedham; no. 534, clearing, Beverly Farms; no. 758, Canton Junction.—In all of these the development is entirely of the *A. alpina* type. Chromosome counts could not be obtained.

A. brainerdii Fernald, no. 270, open pine woods, Tewksbury, Mass.—The development is mostly of the *A. alpina* type, but a single heterotypic anaphase was found (fig. 48). Here the chromosomes are characteristically scattered, and the number counted is 42, which is about the diploid number for *A. brainerdii*, since in this figure it is certain that no chromosomes have been cut away. No other counts could be obtained.

Discussion

TYPES OF APOMIXIS.—The terminology to be used for the various types of apomixis has long been subject to a difference of opinion, there being three main classifications of the various phenomena, given by STRASBURGER (39), ERNST (14); and WINKLER (43, 45). Since WINKLER'S purely morphological classification is the most definite, and has been generally adopted in recent years (ROSENBERG 35), it is the basis for the terminology in this study. According to this terminology the development in the seven species here studied is

diploid parthenogenesis, and where it is completed is, as already noted, of the *Antennaria* type (ROSENBERG 35). This is the most



FIGS. 8-13.—Fig. 8, *A. occidentalis*, abnormal reduction division, diakinesis; fig. 9, heterotypic metaphase; fig. 10, early anaphase; fig. 11, late anaphase; fig. 12, homoeotypic metaphase; fig. 13, hexad. $\times 1800$.

common type of diploid parthenogenesis, and a list of the large number of species possessing it is given by ROSENBERG.

Apospory was noted in *A. neglecta* × *plantaginifolia* (STEBBINS 38), but was not found in any of the species here studied. However, a transition between true apospory and the *Antennaria* type of parthenogenesis is exhibited by *A. parlinii* no. 747, where the original archesporial cell is replaced by an integumentary one, which develops directly into the embryo sac. This replacement of the original archesporial cell by a purely somatic one is found also in *Alchemilla* (MURBECK 25, STRASBURGER 39) and *Oxyria digyna* (EDMAN 13).

Since apomixis generally involves an elimination of the reduction division, or of its resultant haploid cells, from the life cycle of the organism, a comparison of the reduction division and the chromosome constituency of apomictic forms with that in other types of organisms where this division is eliminated or disturbed will clearly throw light on the origin of apomixis.

MEIOTIC IRREGULARITIES.—Irregularities in the reduction division, with their consequent microspore and megaspore degeneration and sterility, are well known to occur regularly in apomictic forms. JUEL (21) noted the complete pollen sterility of *Antennaria alpina* in herbarium specimens, and for that reason supposed it to be of hybrid origin. ROSENBERG (31, 33) noted abnormalities in the various apomictic species of *Hieracium*; while HOLMGREN (19) in *Erigeron*, OSAWA (27) in *Taraxacum*, and WOODWORTH (47, 48) in *Alnus* noted the irregularity of the apomictic as contrasted with the regularity of the reduction division in the sexually reproducing forms.

The irregularities in meiosis may be abnormalities, either in behavior and distribution of the chromosomes or in the condition and activity of the remainder of the nucleus and the cytoplasm. Chromosome irregularities, consisting chiefly of the failure of the chromosomes to pair and their consequent lagging and extrusion into the cytoplasm, are characteristic of interspecific hybrids, as in *Drosera* (ROSENBERG 32), *Crepis* (COLLINS and MANN 9), *Viola* (CLAUSEN 7), and many other genera. The types of irregular division in the pollen mother cells have been well classified by ROSENBERG (34) according to the degree of pairing, as shown by meiosis in the pollen mother cells of species of *Hieracium*. In *Antennaria fallax* and *A. parlinii* the division is of the least irregular, or *H. boreale* type. The dyads in *A. parlinii* are due to a chance bridging of the space

between the daughter chromosome groups in the late heterotypic anaphase by a group of univalents, as described by MATSUDA (23) in *Petunia violacea*, rather than to a true semiheterotypic division.

In the pollen mother cells of *Antennaria canadensis* the *H. laevigatum* type, or semiheterotypic division, occurs. This type, described by ROSENBERG (33) in the apomictic *Hieracium* species, has been found in a number of forms, including many known hybrids and apomictic forms.

The abnormal reduction divisions in the megasporocytes of the parthenogenetic species of *Antennaria* are intermediate between the *boreale* and the *laevigatum* type. Although few or none of the chromosomes form bivalent pairs, and the univalents are scattered throughout the length of the spindle, the "restitution nucleus" never forms, probably due to the length and slenderness of the spindle. A heterotypic division similar to this was described by CHIARUGI (5) in *Artemisia nitida*. This division, however, was not followed by a homoeotypic one; and the homoeotypic division with three spindles, found in the megasporocytes of the parthenogenetic species of *Antennaria*, has not apparently been described in the megasporocytes of any other plants. It does, however, resemble the phenomenon described by SELER (37) in the ovogenesis in the lepidopteran genera *Lymantria*, *Phragmatobia*, and *Orgyria*, and interpreted as chromatin diminution. In these cases, however, a metaphase cell plate is formed, and the chromosomes break and leave some of their chromatin at the equator. This, together with univalents, forms a nucleus during interkinesis.

Extra-chromosomal irregularities, consisting of abnormalities of cytoplasmic behavior, occur in the parthenogenetic species of *Antennaria*. The sporocytes in these forms often have a thin, evacuate cytoplasm, which sometimes behaves anomalously. This is shown most clearly by the changes in position of the spindle and perinuclear zone during the heterotypic division in the parthenogenetic forms. The perinuclear zone was first recognized by BYXBEE (3) in *Lavatera*, and by CANNON (4) in a hybrid cotton, and has since been observed by many workers. In the megasporocyte meiosis of the parthenogenetic species of *Antennaria*, its usual greater abundance at the micropylar end of the spindle during the heterotypic

metaphase, and at the chalazal end during the later stages, accompanied by a corresponding change in position of the spindles in the cytoplasm, indicates considerable shifting of the nuclear and cytoplasmic material back and forth in the cell. This was not observed at all in the normal, sexual species (STEBBINS 38), and is evidence of considerable physical disturbance in the cytoplasm.

Another cytoplasmic phenomenon, found more frequently in the parthenogenetic species of *Antennaria* than in the normal ones, and generally in hybrids and forms with irregular meiosis (DIGBY 12), (HICKS 18), (CHURCH 6), is that of cytomixis, first observed by GATES (17) in *Oenothera gigas*. It is most abundant in the prophase, but CHURCH found it in the heterotypic anaphase of *Panicum sphaerocarpon*, and WOODWORTH (46) in the homoeotypic metaphase of *Betula papyrifera* var. *cordifolia*. Its occurrence as late as the homoeotypic telophase of *A. fallax* is unusual. It has been attributed by ROSENBERG (49) and others to faulty fixation, and by WOODWORTH (49) to pressure against the pollen mother cells during the collection of the material; but its greater abundance in cells with irregular division, those subject to chloralization (SAKAMURA 36) and abnormal temperatures (INARIYAMA 20), indicates that, whether an artifact or not, it reflects in these cases an abnormal state of the cytoplasm, which may often be due to a hybrid condition.

To abnormal conditions in the cells as a whole must be attributed the irregular development of the megaspores formed by the irregular divisions in the parthenogenetic species of *Antennaria*. Cases of the development into an embryo sac of other than the usual chalazal megaspore are found in normal species (AFZELIUS 1), but the development at an approximately equal rate of two megaspores to form different parts of one embryo sac, as in *A. occidentalis*, is distinctly an abnormal condition. A similar case is in the hybrid *Sileneo nebrodensis* × *viscosus* (AFZELIUS), although here one of the two cells completes development by itself into an embryo sac. Apparently the balance in the physiological condition of the megaspores has been so upset by the irregular meiosis that no one of them has the same faculty to develop as has the chalazal megaspore in normal plants.

POLYPLOIDY.—That apomictic forms generally have higher chromosome numbers than their most nearly related normal species has

been recognized by all students of apomixis, such as STRASBURGER (39), ROSENBERG (35), WINKLER (43), and ERNST (14). In *Antennaria* the parthenogenetic species all have higher chromosome numbers than their normal relatives, the diploid numbers varying from 42 to 84, while that in all of the normal species studied is 28.

Polyploid forms are often larger and more robust than their nearest related diploid ones, as is shown by such tetraploid races as *Oenothera gigas* (GATES 16), and *gigas* forms of *Solanum* (WINKLER 44). The polyploid species of *Antennaria* here studied are in general taller and more robust than the sexually reproducing species.

A physiological characteristic of the polyploid species of *Antennaria*, as well as those of *Crepis* (NAWASCHIN 26), and probably other genera, is their slower rate of development. These species of *Antennaria* in the vicinity of Boston bloom about three weeks later than their relatives with a smaller chromosome number.

Polyploid forms are of two main types as regards their nature and origin. Autopolyploids, which have arisen in genetical cultures of several types of plants, such as *Oenothera* (GATES 16), *Solanum* (WINKLER 44), and *Datura* (BELLING and BLAKESLEE 2), contain the chromosome sets of their diploid progenitors duplicated, and do not differ morphologically from them except in their larger size throughout. Allopolyploids have arisen from interspecific hybridization. The first case reported of such a polyploid hybrid is that of *Primula kewensis* (DIGBY 11), and there are many other cases now known, such as those in *Nicotiana* (CLAUSEN and GOODSPEED 8), *Viola* (CLAUSEN 7), *Brassica* × *Raphanus* (KARPECHENKO 22), and *Galeopsis* (MÜNTZING 24). These forms are usually intermediate in their morphological characteristics between their two parents; or they may, as in the case of *Galeopsis tetrahit* (MÜNTZING), have characters distinct from either parent. Allopolyploids, if the chromosomes of their parents are somewhat homologous, may have one or more quadrivalents, with secondary pairing at the heterotypic metaphase, as in *Primula kewensis* (DIGBY 11) and *Rubus rusticanus* var. *inermis* × *R. thyrsiger* (CRANE and DARLINGTON 10); but more frequently such multivalent groups are completely absent.

Whether the parthenogenetic species of *Antennaria* are to be interpreted as auto- or allopolyploids is a problem. *A. fallax* and *A.*

occidentalis resemble, except for their increased size, races of *A. plantaginifolia*; but since they are hexaploids or nearly so with reference to this species, their origin is undoubtedly more complex than that of *Oenothera gigas* and similar forms. Quadrivalent and sexivalent associations are not so general in *A. fallax* as would be expected if the species were a true autopolyploid. Such associations are more frequent in *A. parlinii*, but this species differs from all other known species in other distinctive characteristics besides size. The other parthenogenetic species do not resemble closely any single normal sexual species, and are therefore most likely allopolyploids. Since this type of polyploid has by definition originated through hybridization, and since the parthenogenetic species of *Antennaria* show the characteristics of hybrids and of species of hybrid origin in their reduction divisions, the most plausible theory for their origin is that of ERNST (14) and WINGE (42) for parthenogenetic forms in general, that they have originated through interspecific hybridization.

ORIGIN OF PARTHENOGENETIC SPECIES.—Although the cytological evidence points to a hybrid origin for the parthenogenetic species of *Antennaria*, these species undoubtedly originated many thousands of years ago, and any attempt to discover their parentage is fraught with difficulty.

A. fallax differs from *A. plantaginifolia* only in its larger size throughout, and higher chromosome number. It may have evolved from *A. plantaginifolia* simply by a trebling of the chromosome number, but that hypothesis is improbable, as already noted. More likely *A. fallax* has resulted from a back cross from some hybrid of *A. plantaginifolia* with the parent species, and has thus acquired a preponderance of *A. plantaginifolia* chromosomes. *A. occidentalis* is likewise closely related to *A. plantaginifolia*; but in its obovate, rounded leaves, and its stolons, often longer and more procumbent than in *A. plantaginifolia*, it shows a transition toward *A. neglecta*, or possibly *A. solitaria*, and could have arisen from a back cross from a hybrid between *A. plantaginifolia* and any one of these species to *A. plantaginifolia*.

A. petaloidea, as pointed out by FERNALD (15), resembles *A. neglecta*, differing in its larger, stouter habit and its corymbose inflorescence. The increased size is a result of the trebling of the chro-

mosome number, while the corymbose inflorescence is a character of *A. plantaginifolia*. Furthermore, some plants of *A. neglecta* × *plantaginifolia* in the writer's collection (nos. 495, 514, and 535) resemble *A. petaloidea* except for their reduced size. *A. petaloidea* may therefore be explained as a polyploid, parthenogenetic form which arose from a cross between *A. neglecta* and *A. plantaginifolia*, in the manner of *Primula kewensis* and *Galeopsis tetrahit*, and has spread northward from its place of origin beyond the range of either of its parents.

The other species are more obscure in their affinities. If they are of hybrid origin, one or both of their ultimate parents must now be extinct or absent from the regions that they formerly occupied. Some of the multitudinous varieties and forms must have arisen in one of the following ways.

Hybridization between partially apomictic forms may account for such forms as the series of intermediate races which connect *A. parlinii* with *A. fallax* and *A. occidentalis*. In New England, where staminate plants of *A. parlinii* are almost unknown and those of *A. fallax* are rare, these intermediates are rare or not known; while in the vicinity of Washington, D.C., in Indiana, and the Mississippi Valley, where staminate plants, particularly of *A. parlinii*, are much more abundant, the intermediate races are likewise very abundant and puzzling to the systematist. It seems likely that here hybridization has taken place between *A. parlinii* and its relatives, either through the pollination of the ovules in the "aberrant male" flowers, where the reduction division is not very irregular and functional gametes must be frequently formed, or through fertilization of rare functional gametes formed by an occasional successful reduction division in the pistillate flowers. Such hybridization was produced in the partially apomictic *Hieracium* species by OSTENFELD (28, 29), and is considered by him an important cause of the multiplication of closely related apomictic forms in that genus.

Many varieties and species of a local, restricted range, such as *A. rupicola* Fernald and *A. petaloidea* vars. *noveboracensis* and *subcorymbosa*, have very likely arisen from more widely distributed relatives by the process described in the apomictic species of *Hieracium* by OSTENFELD (29, 30) as apogamic mutation. A possible mecha-

nism for this process is seen in the abnormal first embryo sac division of *A. canadensis* (fig. 4). If the lagging chromosomes in this division failed to reach the proper poles, an egg cell with one or two more or less than the diploid chromosome number would be formed, which, if capable of parthenogenetic development, would produce an offspring with a slightly different chromosome number and hence with different characteristics. Unfortunately, the large numbers of chromosomes in all these forms made it impossible to detect with any certainty slight differences in number between them.

Thus it is seen that in some of the parthenogenetic species of *Antennaria*, both the cytological and morphological evidence points to their hybrid origin; while in the others, the theory that they arose through hybridization a long time ago and have since reproduced themselves by parthenogenesis, while one or both of their parent species have become extinct, at least within their range, is a distinctly probable one. Hence the theory of ERNST and WINGE may be said with some certainty to apply to *Antennaria* as well as to other genera where apomixis is prevalent.

Summary

1. In *Antennaria fallax* the haploid chromosome number is 42, and meiosis in the pollen mother cells is irregular.

2. In the megaspore mother cells of male intersex flowers of *A. fallax*, meiosis is likewise irregular.

3. In *A. parlinii* the haploid chromosome number is also 42, and meiosis in the pollen mother cells is irregular, sometimes failing of completion.

4. In *A. canadensis* meiosis is very irregular, and is frequently a semiheterotypic division, resulting in the formation of dyads of microspores.

5. In the pistillate flowers of all the seven species studied, the embryo sac originates most often directly from an unreduced megaspore mother cell. The egg cell of such an embryo develops directly, without fertilization, into an embryo.

6. In all seven species, the megaspore mother cell occasionally undergoes a very abnormal reduction division. This division usually produces three nuclei instead of two, and the homoeotypic division following it forms a hexad or other polysporic group.

7. Evidence makes it likely that the products of this division generally degenerate.

8. In *A. parlinii* a peculiar type of apospory was found.

9. In *A. canadensis* an abnormal first embryo sac division was found.

10. The approximate diploid chromosome numbers are: *A. fallax* 84; *A. occidentalis* 75-85; *A. parlinii* 84; *A. canadensis* 83-86; *A. petaloidea* 75-80; *A. neodioica* ca. 52; *A. brainerdii* 42.

11. The development in the seven species is diploid parthenogenesis of the *Antennaria alpina* type.

12. In their irregular meiosis, the parthenogenetic species of *Antennaria* resemble other apomictic forms, and known hybrids.

13. Various extra-chromosomal irregularities of meiosis are discussed.

14. The species are all allopolyploids resembling those which have originated through interspecific or intergeneric crossing.

15. Some of the parthenogenetic species are morphologically intermediate between the normal species.

16. The parthenogenetic species have most likely originated from crosses between existing or extinct normal, sexually reproducing species.

The writer wishes to acknowledge with thanks the assistance of Professor E. C. JEFFREY, who suggested the problem and whose advice and technical direction have been invaluable throughout the work. Thanks are also due to Dr. S. F. BLAKE for assistance in obtaining material of the species growing in the vicinity of Washington, D.C.

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EXPLANATION OF PLATES II, III

PLATE II

All figures, $\times 2400$.

FIG. 14.—*Antennaria fallax*, microsporogenesis, heterotypic metaphase.

FIG. 15.—Same, polar view showing 42 bivalent chromosomes and 4 univalents (in stipple).

FIG. 16.—Heterotypic anaphase showing laggards and cytoplasmic connections with a neighboring cell.

FIG. 17.—Homoeotypic metaphase.

FIG. 18.—Homoeotypic telophase showing cytomixis.

FIG. 19.—*A. parlinii*, microsporogenesis, heterotypic metaphase.

FIG. 20.—Same, showing 37 bivalent and 10 univalent chromosomes.

FIG. 21.—"Restitution nucleus" at interkinesis of microsporogenesis.

FIG. 22.—Polysporic heptad.

FIG. 23.—"Dyad" with two extra microcytes.

FIG. 24.—*A. canadensis*, early semiheterotypic anaphase of microsporogenesis.

FIG. 25.—Late semiheterotypic anaphase, showing bent spindle.

FIG. 26.—Restitution nucleus.

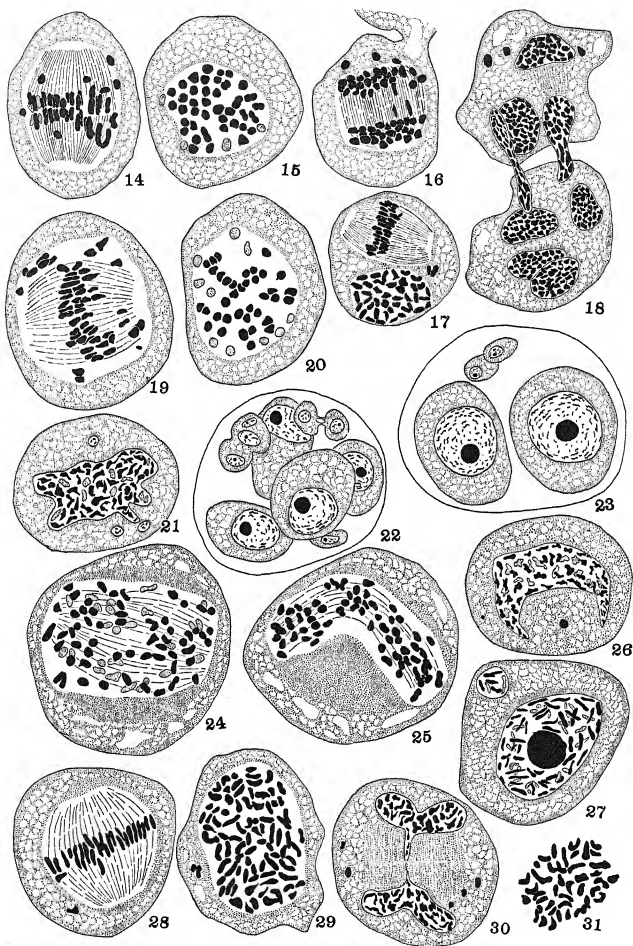
FIG. 27.—Same, later stage showing splitting of chromosomes; extra micro-nucleus present.

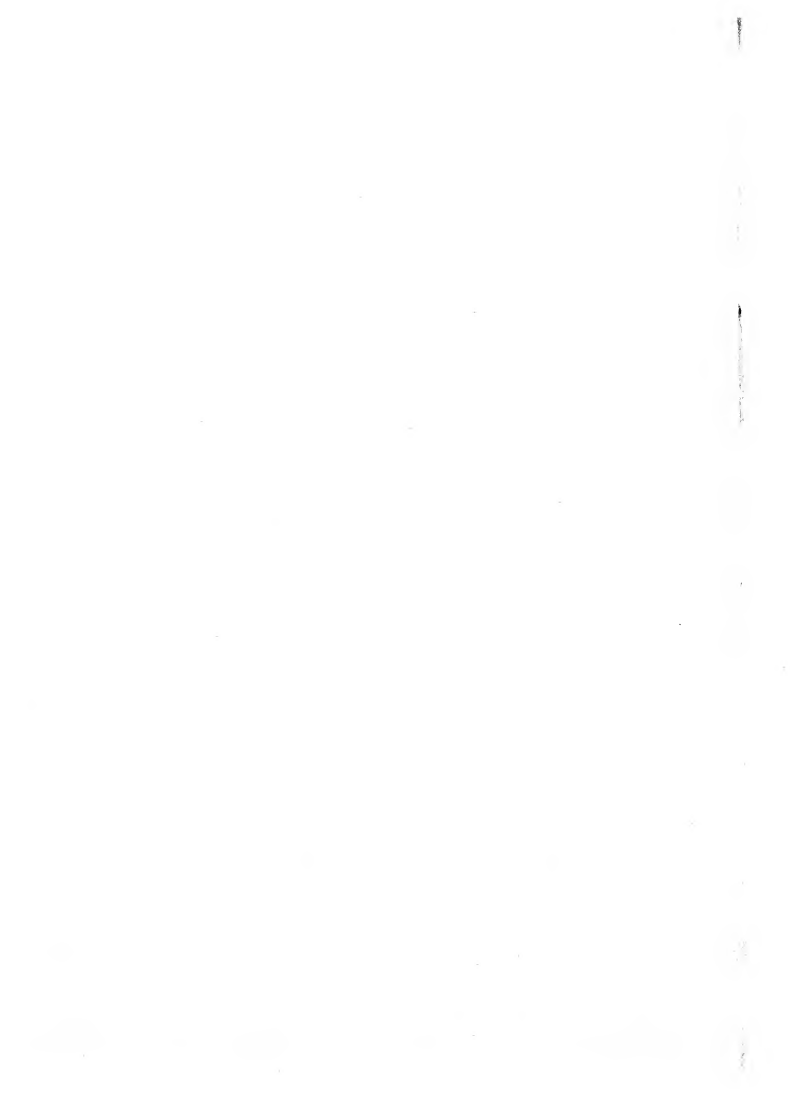
FIG. 28.—"Homoeotypic" division of restitution nucleus, metaphase.

FIG. 29.—Same, polar view.

FIG. 30.—Telophase of same.

FIG. 31.—Cell plate, polar view, of regular homoeotypic metaphase.





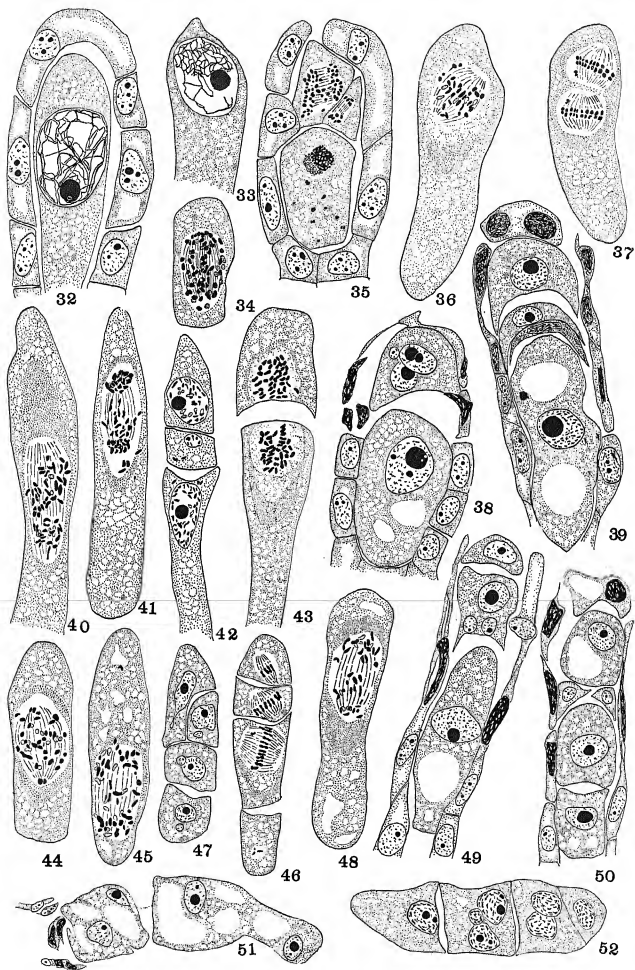




PLATE III

Figure 51, $\times 600$; all others $\times 1200$.

FIG. 32.—*Antennaria fallax*, prophase of abnormal reduction division showing spireme.

FIG. 33.—*A. parlinii*, same showing synizesis.

FIG. 34.—*A. fallax*, abnormal reduction division, heterotypic metaphase.

FIG. 35.—Homoeotypic anaphase of same.

FIG. 36.—Megaspороgenesis in "aberrant male" flowers, heterotypic metaphase.

FIG. 37.—Homoeotypic metaphase of same.

FIG. 38.—Tetrad in "aberrant male" flowers.

FIG. 39.—*A. canadensis*, tetrad of megaspores.

FIG. 40.—*A. petaloidea*, heterotypic metaphase.

FIG. 41.—Heterotypic anaphase.

FIG. 42.—Interkinesis, showing three cells.

FIG. 43.—Homoeotypic metaphase.

FIG. 44.—*A. neodioica*, heterotypic metaphase.

FIG. 45.—Heterotypic anaphase.

FIG. 46.—Homoeotypic metaphase showing four cells and three spindles.

FIG. 47.—Tetrad with microcytes.

FIG. 48.—*A. brainerdii*, heterotypic early anaphase.

FIG. 49.—*A. occidentalis*, pentad.

FIG. 50.—Old hexad, two cells developing.

FIG. 51.—Abnormal embryo sac.

FIG. 52.—*A. parlinii*, tetrad.

METHODS FOR CONTROLLING THE ENVIRONMENT OF GREENHOUSE PLANTS¹

P. W. WILSON AND C. E. GEORGI

(WITH THREE FIGURES)

Introduction

In all physiological experiments on plants made in the greenhouse, it is desirable to control, in so far as is possible, the environment under which the plants are grown. Although this problem doubtless arises frequently among workers in plant physiology, agronomy, and plant chemistry, there are surprisingly few contributions concerned with simple and comparatively inexpensive means of controlling such an environment.

In most physiological and chemical studies on plants, there has been a tendency either to ignore entirely the possible effects of changes in light, humidity, temperature, etc., or to control these within very narrow limits by means of elaborate and often expensive equipment. The second method is certainly to be preferred, but it is not always available to the majority of investigators in plant physiology. The technique developed at the Boyce Thompson Institute (2, 3) for maintaining constant conditions for the plants is desirable but not practicable for most investigators, both from the financial and technical points of view. The less expensive constant-condition chambers of the type described by DAVIS and HOAGLAND (4), TOTTINGHAM (19), and STEINBERG (17) have the objection that the space controlled is limited and therefore cannot be used for extensive experiments.

It would seem highly desirable that a technique be developed suitable for large-scale experiments to be used in greenhouse studies. Such a technique would use as a basis the results already obtained in the more elaborate arrangements referred to, but would seek to

¹ Herman Frasch Foundation in Agricultural Chemistry, Paper no. 46. Contribution from the departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

apply them to actual working conditions in the greenhouse. As examples of the possibilities of this application to the control of rooms capable of large-scale experiments may be cited the constant-condition rooms described by SANDE BAKHUYZEN (14) or HARVEY (7). While the exact control obtained in the constant-condition chambers, or even in the larger rooms, could not be expected in greenhouse work, at least a partial control over the environment might be achieved. It must be remembered also that any efforts to regulate light, atmosphere, humidity, and temperature lead to an appreciation of the influence of these factors upon the results, and a more nearly correct interpretation of the data is therefore possible.

Experimentation

GREENHOUSE.—The greenhouse used in this work is a unit in a series that face north; at the southern end it is joined to the headhouse, which partially shades this end. The unit is 36 feet long and 22 feet wide. Each side is lined with a bench 3.5 feet wide and 3 feet in height. In the center of the house and separated by a cement walk 3 feet wide, are two benches that extend from the north end for 15 feet and are 4 feet wide and 4 feet in height. The remainder of the length of the greenhouse in the center is occupied by benches of the same dimensions, except that the height is only 3 feet. The benches are so constructed that there is a minimum of obstruction under them, thus allowing free circulation of air; the steam pipes used to heat the house in the winter are located under the side benches. On the top of each bench is a 2×4 railing; this forms a shallow bed which is lined with tar paper and filled with wet sand in which are placed the plant cultures. Sand is also placed under the beds and is kept moist by frequent sprinkling, as are the concrete walks that surround the beds. Solid $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ is kept on the wet sand beds and on top and under the benches, in order to prevent the growth of algae. At the extreme south end of the house the side benches are not covered with sand, and a space 5 feet in length is reserved for laboratory work. Here the plant cultures are put up and certain routine chemical analyses made.

GAS MIXTURES.—In many physiological problems it is necessary to regulate the partial pressures of the gases supplied the plants,

especially the $p\text{CO}_2$ and $p\text{O}_2$. The method to be described was developed for study of small leguminous plants, but it could be extended to larger species by modification of the container for the plant cultures. Since a majority of studies concerned with the gase-

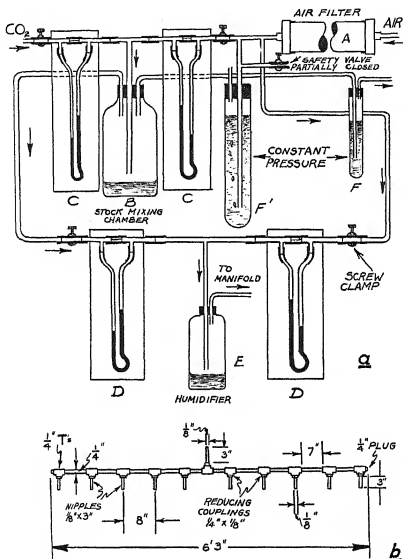


FIG. 1.—Diagram of apparatus for regulation of $p\text{CO}_2$ in atmosphere

ous environment of the plant deal with the effect of $p\text{CO}_2$, a detailed description will be given of the method used to regulate the partial pressure of this gas.

A diagram of the system used to supply atmospheres of various CO_2 content is shown in figure 1. Air from a compressed air-line is passed over concentrated H_2SO_4 , then through a 2.5-inch pipe (A)

32 inches in length which has been filled with cotton and sterilized. The air current is then divided and a measured quantity combined in the stock mixing chamber (*B*) with a known volume of CO_2 obtained from a commercial cylinder. The volume of each component of the resulting mixture is measured by means of the flow meters (*C*) which have been standardized to deliver a known volume of gas per unit of time for any given difference in pressure registered. In the mixing chamber is kept a saturated solution of NaCl which aids in regulating the humidity of the gas mixture. The air plus CO_2 is then mixed with ordinary air, as indicated in figure 1 *a* (flowmeters *D*) to make an atmosphere of the desired CO_2 content. The mixing is done in the humidifier (*E*), which contains a saturated NaCl solution, and then passes to a manifold for distribution among the plant cultures.

For example, an air- CO_2 mixture which contains 0.8 per cent CO_2 is made by passing air into *B* at the rate of 5000 cc. per minute and CO_2 at the rate of 40 cc. per minute. An atmosphere, which has a CO_2 content of 0.2 per cent, can now be made by mixing in *E* 620 cc. of air (0.03 per cent CO_2) and 180 cc. of the 0.8 per cent CO_2 mixture. The 0.2 per cent mixture is thus delivered to the manifold at the rate of 800 cc. per minute; it is obvious that any other rate could be supplied by modification of the volumes of the two components used. In order to simplify figure 1, only one set of flowmeters for supplying a manifold is indicated, but actually five or six can be taken care of by one stock mixing chamber. More stock mixture (0.8 per cent CO_2) is made than is needed for the cultures, and the excess is carried from the greenhouse through the constant pressure device (*F*).

For distributing the gas mixtures to the manifolds 0.25-inch copper tubing is very satisfactory, as it is inexpensive, flexible, and durable; glass tubing can be used but it is not so convenient as the copper tubing. A heavy-walled red rubber tubing is used for the necessary rubber connections; a high grade will last at least six months in greenhouse use. For delivering the gas mixture to the plants, a manifold of the type detailed in figure 1 *b* can readily be made. The construction of the flowmeter is shown in detail in figure 2 *a*. The glass-stoppered drying tube is used to regulate the flow of gas and to collect excess moisture in the gas; this tube is not absolutely necessary since ordinary screw clamps are satisfactory for regulation, as indi-

cated in figure 1a. The capillary tubing varies in size from 0.1 to 2 mm., depending upon the rate of flow desired. Since the proper size will also depend upon the pressure at which the gases are delivered (height of water in F and F_r), the correct size for a given rate

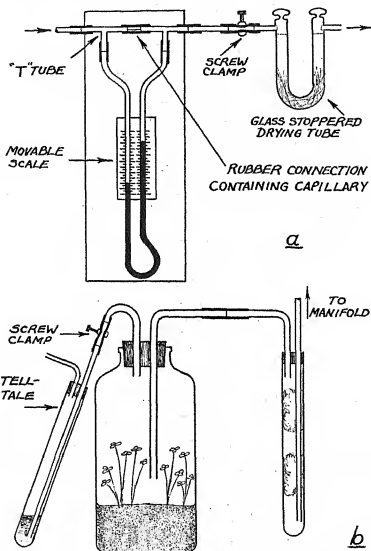


FIG. 2.—Details of flowmeter and culture bottle, approximately one-third actual size.

of flow is best determined by trial. The liquid used for an indicator in the flowmeters is a matter of choice, but one with a low vapor pressure at ordinary temperatures is desirable; n-butyl phthalate colored with methyl red dye is very satisfactory.

To standardize a flowmeter, the gas mixture which it is to measure is passed through the capillary tube under a pressure sufficient

to maintain a given difference in the levels of the liquid in the arms of the U-tube; the time required for a known volume of the gas to pass through is determined and is calculated to cc. per minute. This is repeated for several readings of the flowmeter, and from these data a calibration curve is constructed. Since for small differences in pressure the rate of flow through the capillary is a straight line function of the difference in pressure, three determinations usually will suffice for a calibration.

The plants are grown in 64-oz. bottles of clear flint glass in which a substrate of Crone's agar or sand plus Crone's solution is used. The details for planting under bacteriologically controlled conditions have been described in a previous publication (8). After planting, the cotton plug is replaced by the apparatus shown with the bottle in figure 2 *b*; this unit is sterilized separately in wrapping paper just before use. The gas from the manifold is filtered through sterile cotton before it passes over the plant. The rate of flow through each bottle on a given manifold is kept fairly uniform by regulation with a screw clamp on the tell-tale; the latter contains HgCl_2 solution (1:1000). The method just described is applicable only to experiments in which plants are grown in a closed system; if this is not desirable, a modification can be used. The plants are grown on sand in 64-oz. bottles which are left open to the air; the gas mixture from the manifold passes through a tell-tale, and is then led by a bent glass tube to the surface of the sand where it must diffuse among the plants before escaping into the greenhouse. The use of a bottle prevents air currents from mixing the added CO_2 with the surrounding air as soon as it is delivered to the plants. With clover and alfalfa this system was very satisfactory, as the plants grew out of the tops of the bottles to a large size and were in every way comparable with plants grown in open sand pots.

ILLUMINATION.—If considerable work is done during the winter months, the question of sufficient light for plants is a most important problem. The lack of sunlight in this latitude becomes very acute from November until March, as is indicated by the meteorological data for this period. The average daily sunlight for these months at Madison, Wisconsin, is: November 3.9 hours, December 3.3 hours, January 4.0 hours, and February 5.2 hours. Also the effect of the

lack of sunshine is even greater than these data indicate, since the average values are given; a perusal of the daily reports for these months shows that there are many days in which no sunshine appears. This leads to an interrupted, erratic growth of plants, unless the deficiency in light is compensated in some way. The use of artificial lights to supplement or replace sunlight in plant experiments has been investigated by many workers and the results have been most encouraging. SHIRLEY (16) has recently reviewed the various sources available, with comments on their efficacy; representative papers concerned with the effect on plant growth of artificial illumination are those of HARVEY (7), DAVIS and HOAGLAND (4), TJEBBES and UPHOF (18), SHIRLEY (15), and HARDER, KEPPLER, and REUSS (6).

The question of the type of lamp to use as a source of artificial light is a difficult one; 100 to 1500-watt lights have been used with varying success, and the decision usually rests upon considerations other than mere intensity and quality.

A study of the papers dealing with growth of plants under artificial lights indicates that satisfactory results are obtained with an intensity from 400 to 1000 foot-candles; however, in most cases the electric lamps were used as the sole source of radiant energy, so that somewhat lower intensities would probably be satisfactory if the lights are employed to supplement and not to replace sunlight. Table I, constructed from data given by LUCKIESH (12), gives the light characteristics of Mazda lamps of various watt ratings compared with sunlight. The intensities were calculated on the assumption that reflecting devices were available which would allow 75 per cent of the total radiation to be spread uniformly over a horizontal space 4 square feet in area. These data show that as the watt rating increases, the lamp becomes more efficient (lumens per watt) and the distribution of energy more favorable for plant growth; that is, it is displaced toward the lower wave lengths.

In spite of these characteristics, the use of high wattage lamps is not always feasible in greenhouses because of the expense and heating effects. The heat production (infra-red radiation) is given in the last two columns of table I; the last column gives the rate at which heat is supplied to an area of 4 square feet on the assumption that the infra-red radiation is reflected and transmitted to the same de-

gree as are the visible wave lengths. It is apparent from this table that unless means are available to remove the infra-red radiation (for example, water screens), it will be necessary to keep the high rating lamps farther from the plants and to spread the total energy flux over a large area. If this is done, the visible illumination is likewise decreased in intensity so that there is usually a limit in ordinary greenhouse practice to the intensity of light that can be supplied the plants from electric lamps. For example, if the radiation from a

TABLE I
LIGHT CHARACTERISTICS OF MAZDA LAMPS AND SUNLIGHT

SOURCE OF LIGHT	COLOR TEMPERATURE (° K)	LUMINOUS EFFICIENCY (LUMENS PER WATT)	INTENSITY* (FT.-CANDLES)	PERCENTAGE DISTRIBUTION OF ENERGY†				HEAT PRODUCTION	
				0-3000 Å	3000-4000 Å	4000-7600 Å	7600 Å	PER FT.-CANDLE (CAL./MIN./CM².)	TOTAL ON 4 SQ. FT. (CAL./MIN.)
I. MAZDA LAMPS									
50 watt...	2670	10.0	94	0.002	0.85	9.2	90.7	1.40 × 10 ⁻³	49
100 " ...	2740	12.9	242	0.003	0.11	10.1	89.8	1.06 "	975
200 " ...	2810	15.2	566	0.004	0.14	11.1	88.7	0.90 "	1900
500 " ...	2920	18.1	1700	0.008	0.20	12.7	87.1	0.74 "	4670
II. SUNLIGHT‡									
June	5000-6000	8540	0.022§	3.9§	43.7	52.4	0.094 "	2970
February		4840	0.006	2.8	42.1	55.1	0.154 "	2820
December		2415	0.0005	1.6	39.1	59.3	0.281 "	2520

* Values for the Mazda lamps were calculated on the assumption that reflection is 75 per cent efficient and that the total radiation is uniformly spread on a horizontal surface, 4 sq. ft. in area.

† Per cent of total radiation.

‡ Data are for sunlight in Cleveland, Ohio.

§ Values for the sun's radiation are for 2900-3100 Å and 3100-4000 Å respectively.

500-watt lamp is distributed over an area such that the infra-red radiation supplied per unit area per unit of time is equal to that of a 200-watt lamp, the visible light supplied will be about 20 per cent higher in the case of the 500-watt lamp. This calculation, however, is based on the assumption that the reflection and transmission of the various wave lengths are the same, and since this is not true it must be corrected. The longer wave lengths are more readily transmitted through air and reflected from surfaces; therefore the value is too high. In order to accomplish this distribution of energy in actual practice, it would likely be necessary to use larger reflectors and to

maintain the 500-watt lamps farther from the area to be illuminated. Because of the differences in transmission and reflection of the various wave lengths, it is doubtful whether the intensity of the visible light would be very different in the use of the two lamps under these conditions. It is apparent that a choice must be made between the use of many lamps of low wattage or fewer lamps of a higher rating. The choice will usually resolve itself into a consideration of the expense of installation and the upkeep of the various types.

In our own work, 200 and 300-watt lamps have been found to be satisfactory as a supplement to sunlight during the winter months. Above each of the center beds are suspended two 1-inch conduits about 1 foot from either side of the bench. Every 4 feet in the conduits there is fitted a receptacle with two outlets for the plugs of lamp cords. The lamps are suspended from the conduits by a light-weight chain which is attached to the shade by means of a yoke, also made of chain. These chain supports are adjustable, so that the position or the height of the lamp can be varied at will. The conical reflecting shades for the 200-watt lamps are 10 inches in diameter at the base and about 3 inches in height; a collar 1.5 inches in height and 2 inches in diameter is fitted to the top of the cone to facilitate attachment to the lamp socket. The shades are of bright tin and are sprayed with a clear lacquer to preserve the reflecting surface. The two middle beds are provided with twenty-eight 200-watt lamps suspended 12 to 18 inches above the plant cultures; each lamp provides illumination for about 4 square feet, and the lights are moved about until the illumination over a bed is as uniform as possible. For the illumination of the side beds, 300-watt lamps 4 feet apart and about 30 inches above the beds are used; these lights are screwed directly into the receptacles, which can be moved through an angle of 45° . In this way a more uniform distribution of the light over the bed is obtained, but the illumination is not nearly so uniform over these side beds as over the center ones. A larger reflector is also necessary; a conical shade 14 inches in diameter at the base and 5.5 inches in height is used.

The advantage of this system of lighting is the flexibility of the set-up. The lamps can be moved whenever necessary, and in this way the intensity of the illumination can be kept fairly constant over the plants. The lights are used between 8 A.M. and 9 P.M. on dull,

cloudy days, but on bright days they are used only after the sun has declined to a point where artificial illumination becomes an effective part of the total radiation. Since the lights are fitted to the receptacles with pull-out plugs, they can easily be removed during the months in which natural illumination is sufficient; that is, from the middle of April to the first of October.

TEMPERATURE.—One of the most difficult factors to control in greenhouse work is temperature. This is especially true during the summer, since most systems of ventilation are useless at this time unless refrigeration of the incoming air is possible. During the winter, steam heat, controlled by a bimetallic regulator, is used to keep the temperature about 25° C. When the lights are in use the temperature rises, but by opening the ventilators in the roof slightly and using large electric fans to keep the air circulated, it is possible to maintain the temperature between 25° and 30° C. without much difficulty.

In early spring, an unusually bright day may send the temperature up 10°–15° C. unless there are means to reduce the intensity of the sunlight coming through the glass. To avoid this overheating it has been found advisable to provide shades for the roof. These are made of "longcloth," 3 feet wide and 12.5 feet in length, and are fastened by means of brass eyelets to heavy copper wire. The shades are easily made and the materials are inexpensive; they are very effective in lowering the intensity of light whenever it becomes so great that the temperature of the greenhouse cannot be kept below 30° C. The shades have an advantage over whitewash in that it is necessary to shade only that part of the greenhouse which is receiving direct illumination, and hence reflected light and skylight can be utilized entirely. As spring advances, however, it is necessary to apply whitewash to the roof of the greenhouse in successive coats; by July, a fairly heavy protection of whitewash has been applied to the upper part of the roof which receives the intense radiation of the midday sun, and a less heavy coat to the remainder. Of course, the application of the whitewash lowers the intensity of illumination; but, because of the long days at this time of the year, small leguminous plants, as clover and alfalfa, grow rapidly and with no evidence of lack of light. For larger plants, as peas and soy beans, it has been found advisable to use the less easily controlled outside cold-frames.

During the spring and summer months the beds and walks are kept wet; 16-inch fans keep the air circulated, which aids in lowering the temperature in the sand beds by rapid evaporation of their moisture. Removal of several panes of glass from the roof at the eaves allows a good circulation of air to be maintained throughout the greenhouse.

ROUTINE CONTROL.—The methods described for the regulation of the various factors require a certain amount of routine checking and analyses for their successful operation. Every two hours during the day an inspection of the house is made, at which time the flow of the gases is checked; the temperature, humidity, nature of illumination, and other observations are recorded in a log-book. This routine check starts at 8 A.M. in the winter and 7 A.M. in the summer; at these hours the gas mixtures are started, the lights turned on if necessary, and the beds and walks watered. The last inspection is made at 9 P.M., at which time the gas mixtures are stopped, the lights are turned off, and the ventilators adjusted. Temperature readings are taken in various parts of the greenhouse on thermometers fitted in the rubber stoppers of the type of bottle shown in figure 2 *b*; the bulb of the thermometer is blackened in order that the indicated temperature will be a maximum. The temperature is maintained between 25° and 30° C. in so far as is possible; if the readings are not within these limits, the ventilators and fans are adjusted to remedy the excess or deficiency.

There is no provision for control of humidity in the greenhouse proper, but readings are taken with a Tycos hygroscope in order to take corrective means when needed. Usually the humidity is 60 to 70 per cent, and is maintained fairly constant by the watering and the air circulation; it is easier to control during the winter than during the hot, moist summer. The humidity of the gas mixture is regulated to a certain extent by passing the gases over saturated NaCl solution, but this is not too successful, since moisture often collects on the inside of the bottles used to grow the plants. The composition of the gas mixtures is rather easy to regulate, since once adjusted, the rate of flow of the various gases is maintained from 2 to 3 hours without change.

At the time of each inspection, a note is made in the log-book in re-

gard to sunlight and whether or not electric lamps are in use; in this way the total number of hours of artificial illumination can be calculated for a given experiment. Meteorological data from the local Weather Bureau are added to these observations at the end of each month. If possible, routine determinations of the total illumination received at various times of the day might prove of value. The various types of apparatus for measuring light have been discussed by SHIRLEY (16), with comment on the accuracy and technical skill required for manipulation. In control of this nature great accuracy is not required, since the intensity of light will vary in different parts of the greenhouse, especially when the illumination from the sun is included. Ease and speed of manipulation are much more important than an error of less than 1 to 2 per cent. A photometer based on the action of a photoelectric cell has recently been put on the market by the Weston Electric Company; it is claimed that it is accurate to 5 per cent when used to measure the illumination from the sun or an electric lamp, and that it is very easy to operate. One has been ordered and will be tested during the next growing season. Measurements made with a photometer of the illumination from the electric lights alone indicated an intensity of 250 to 300 foot-candles at the level of growth of the plants. If no special apparatus for the measurement of light is available, indirect methods such as those described by LIVINGSTON (11) might be a desirable substitute.

In addition to these routine observations, certain periodic analyses might be made; for example, CO_2 in the gas mixture and ultraviolet radiation. A simple method for the determination of CO_2 in a mixture has been described elsewhere (21). Air plus CO_2 is bubbled through a solution of NaHCO_3 of known strength; the pH of the solution is determined by a glass electrode, or colorimetrically; the pCO_2 in the air is determined from a calibration curve by means of this pH.

In addition to the visible light measurements, some idea of the quantity of radiation of lower wave lengths in the illumination is often required. Since the ultraviolet and near ultraviolet light will vary with the period of the year and with the type of artificial light used, a quick convenient method for estimation is desirable for periodic analysis. LUCKIESH (13) has reviewed the many methods avail-

able for estimation of ultraviolet radiation; these are based on some specific effect of light of low wave length, such as its photochemical, physical, photogenic, physiological, or photoelectrical action. Accurate measurements require the use of the thermopile, bolometer, or the radiometer with suitable filters, but these methods are too time-consuming and exacting for routine analysis. The most promising methods for general use appear to be those based on the photochemical effects of the ultraviolet and near ultraviolet rays; these are suitable provided it is desired to determine the ultraviolet and violet radiation as a whole rather than any particular narrow range of wave lengths. None of the photochemical methods is specific for a given region (for example, less than 3000 \AA°), but will overlap, with a changing quantum efficiency, into higher wave lengths. Nevertheless for control work these methods have much to recommend them, provided they are used with full cognizance of the limitations.

A variety of photochemical reactions for the measurements of ultraviolet light have been proposed, including the photochemical decomposition of methylene blue (9), of quinone (5), triphenyl methane dyes (20), and uranyl oxalate (1, 10). The last reaction has been investigated very thoroughly in regard to the effect of different wave lengths, the temperature coefficient and quantum yields, and for this reason can be used with greater confidence than the other reactions. The technique of the estimation has been described in detail by LEIGHTON and FORBES (10). These workers showed that wave lengths up to 4900 \AA° decompose uranyl oxalate, but that the percentage of radiation absorbed decreases rapidly for wave lengths above 4000 \AA° . They also found that the temperature coefficient of the reaction is only 1.03, and that the quantum yield varies from 0.49 to 0.60 with an average of 0.55. These data indicate that with polychromatic light the method will measure not only the ultraviolet, but to a certain extent the violet and blue; that is, above 4000 \AA° . This latter is not a serious objection to control analyses, but it should be kept in mind when making measurements; for example, the light from a 200-watt lamp gives some decomposition of the uranyl oxalate, but this is probably due to the presence of wave lengths above 4000 \AA° . For greenhouse work, special containers for the uranyl oxalate during exposure can be made from small weighing

bottles; these are covered with tinfoil on the outside to reflect the radiation not absorbed and are filled with sealing wax until the surface of 10 cc. of solution is almost level with the top of the bottle. The surface exposed should be about 400 mm.², so that the depth of liquid is 2.5 cm. This depth is necessary, as ANDERSON and ROBINSON (1) have shown that not all radiation is absorbed unless the thickness of the solution exposed is 1.5–2.0 cm. After 30 to 60 minutes' exposure, the solution in the weighing bottle is washed into a 25 × 200 mm. pyrex test-tube, heated at 80° C. for 5–10 minutes, then titrated against 0.02N KMnO₄. Blanks on the reagents are made by the same procedure, except that the containers are kept in the dark for 30 minutes. In our analyses, the intensity of the radiation was calculated on the assumption that the average wave length measured is 3600 Å; this calculation is arbitrary, and for comparative purposes the results expressed in cc. of KMnO₄ decomposed per unit of time are satisfactory.

The method was tested in the greenhouse by measuring the radiation from a General Electric sun-lamp. It was found that very good checks could be obtained on replicate determinations, and that the intensity measured was independent of the time of exposure; for example, five determinations were made on samples exposed from 45 to 240 minutes and the average intensity found was 68 ± 5.9 ergs per sec. per mm.² In another determination in which the solutions were exposed for 30 to 120 minutes to a higher intensity from the sun-lamp, the average value found was 86 ± 3.7 . Part of the variation noted is due to inability to place the solutions so that the radiation from the lamp is the same on all surfaces. When the sun-lamp was suspended 84 cm. from the uranyl oxalate solution, an intensity of 39 ergs per sec. per mm.² was observed; while a 300-watt lamp at a distance of 52 cm. had an intensity of 13 ergs per sec. per mm.² Placing the solutions inside the flint glass bottle used for growing the plant cultures lowered the intensity of these low wave lengths to 50 per cent of the values on the outside.

Discussion

The partial control of greenhouse lighting, temperature, gas environment, and humidity here described is necessarily only relative;

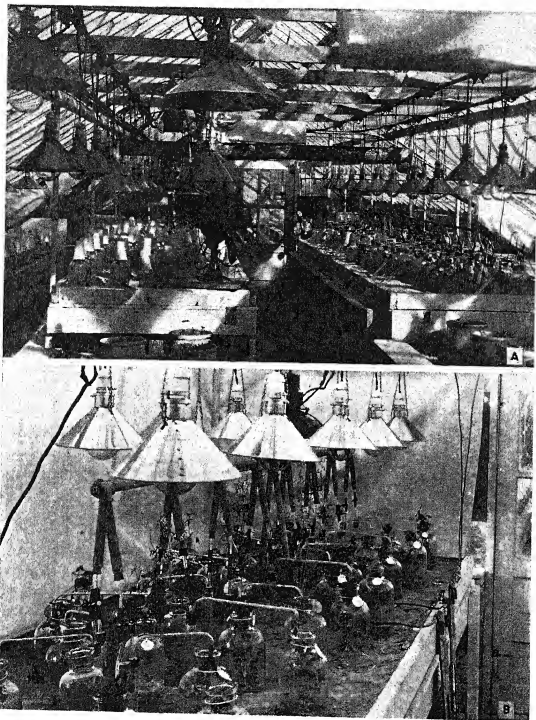


FIG. 3.—*A*, general view of greenhouse; *B*, apparatus for increasing $p\text{CO}_2$ supplied to plants grown in open.

but by a few routine observations and chemical determinations, it is possible to evaluate the environment of a particular experiment to a higher degree of accuracy than is usually obtained in ordinary greenhouse work. In figure 3 *A* is shown a general view of the greenhouse in which these methods of regulation have been applied. Figure 3 *B* is a close view of the system used to grow plants in an open system supplied with additional CO_2 . The fine appearance of the plants is obvious in this photograph. The red clover plants, which were about 6 weeks old at the time of the picture, were placed in the greenhouse December 13, and their growth had taken place during a period in which the average daily sunlight was only 2.5 hours. The sweet clover plants which are 3 weeks older are in bloom; about a week after the picture was taken these plants set seed.

Of course the technique touches only a few of the many variable factors which are important in the study of plants, but it is felt that contributions from other workers with like problems will eventually result in a system of greenhouse control that is within the reach of most of the workers in plant physiology or plant chemistry. While the control achieved can never be expected to be more than a first approximation to that developed for small constant-environment chambers, or the larger expensive greenhouse units, it will have the advantage of more universal application.

Summary

1. Methods are described for the partial regulation of the gas, temperature, humidity, and light environment of greenhouse plants. The partial pressure of CO_2 in the gas mixture supplied the plants is regulated by mixing air with CO_2 from a cylinder; the volume of each constituent is measured with a standardized flowmeter. The humidity of the air- CO_2 mixtures is partially controlled by passing these over a saturated solution of sodium chloride.

2. To supplement the natural illumination from the sun with artificial lighting in the winter months, 200 and 300-watt Mazda lamps, equipped with bright tin reflectors, are provided. The lamps are kept 12 to 18 inches above the cultures, and are so distributed that the intensity of the light received by plants is about 250 foot-candles.

3. The temperature of the greenhouse is regulated by means of steam heat, ventilators, electric fans, and frequent sprinkling of the sand beds and walks. In order to reduce the intensity of the sunlight during the spring, the roof of the greenhouse is equipped with shades; during the summer a heavy coat of whitewash is applied to the roof. Commercial copper sulphate is distributed throughout the house to prevent the growth of algae.

4. Routine analyses are made periodically and recorded in a log-book; these include meteorological observations, estimation of CO_2 , and determination of ultraviolet light.

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FORMATION OF CALLUS KNOTS ON APPLE GRAFTS AS RELATED TO THE HISTOLOGY OF THE GRAFT UNION¹

JOHN E. SASS

(WITH PLATES IV-VII)

Introduction

The common occurrence of gall-like overgrowths on apple nursery stock is a source of considerable economic loss to nurserymen. It is now generally known that a certain type of overgrowth is caused by an organism, *Pseudomonas tumefaciens* Sm. and Town. Evidence has been accumulating, however, to show that all overgrowths on grafted apple nursery stock are not necessarily of bacterial origin. As recently summarized and augmented by MUNCIE and SUIT (9), it seems that the best isolation technique recovers a pathogen in less than 1 per cent of the overgrowths examined. The great majority of the overgrowths encountered in the nurseries apparently develop in the absence of a pathogen.

Our limited knowledge of the anatomy of true (bacterial) crown-gall and non-bacterial overgrowths has accumulated largely as a secondary issue of bacteriological investigations. It is clear that the tissues of both types of overgrowth consist of proliferated tissues of the plant. In the case of crown-gall, the excessive proliferation of the host tissues is brought about by the presence of bacteria in the tissues. In view of the fact that non-pathogenic overgrowths are most common on poorly-healed grafts (RIKER and KEITT 12, MUNCIE and SUIT 9), the cause of excessive callusing has been ascribed to some disturbance in the healing process.

HERSE (5) made a detailed study of the histology of healing in splice grafts of apple. He showed clearly that the respective calli of

¹ This work has been carried out in connection with the crown-gall project in which the Crop Protection Institute of the National Research Council, the University of Wisconsin, the Office of Mycology and Disease Survey of the United States Department of Agriculture, and Iowa State College are cooperating. The manuscript has been critically read by Dr. I. E. MELHUS.

the stock and scion coalesce by the mingling of spongy callus cells, and that in well-matched grafts a new cambium appears, bridging the intervening callus between the members in about ten weeks. In poorly-matched grafts, instead of a straight bridging layer of cambium, a recurved cambial extension is differentiated from the stock and scion respectively. Subsequently there are formed on the margin of each member of the graft irregular and unconnected rolls of vascular tissue, which may constitute more or less permanent barriers to union. An excellent review of the literature on the healing of wounds and graft unions has recently been published by BRADFORD and SITTON (2), making repetition in this paper unnecessary.

Materials and methods

Scion wood for the grafts used in this work was taken from Wealthy trees near Ames, Iowa, and the roots used as stocks were from one-year-old Kansas-grown French crab seedlings. Piece-root tongue grafts were made by Dr. J. H. MUNCIE, in a manner similar to nursery practice except that the cut surfaces were kept reasonably clean, and most of the joints were fitted accurately. Standard waxed twine was used for wrapping. Only the stated combination of stock and scion was used, and one type of graft was made. The data presented in this paper were obtained entirely from this material. The grafts were promptly packed in sterilized peat, in which they were stored at about 18° C. until used. Representative grafts and growing trees were collected at intervals and prepared for histological study.

For the study of the early stages of callusing, thin slices were removed from the callusing surfaces of the separated stock and scion. Just enough wood was included to furnish orientation. The graft unions were removed intact as soon as they had callused enough for the members to stay together during handling. In order to facilitate penetration by reagents, superfluous wood was cut away, leaving only the callusing surfaces involved in the healing process.

Acetic-formalin-alcohol was used as a killing agent. The younger stages were imbedded in paraffin, and the complete grafts were handled in celloidin. Sectioning of the more woody paraffin material was greatly facilitated by treating the blocks, trimmed ready for

cutting, in water at 40° C. for two or three days. After this treatment the paraffin becomes translucent, aiding in orienting in the microtome, and the sapwood and attached bark are sectioned easily. Mayer's haemalum and safranin were found to be the most useful stains.

Early stages of callus formation

The type of graft used in this study is made during the winter, when the tissues are in the dormant condition. At this time there is a clear-cut demarcation between the matured wood of the past season and the tissues of the bark. In this paper the term bark is used to include all tissues outside of a more or less distinct woody cylinder. Dormancy is broken very soon after the grafts are made, for mitotic figures were evident in the tissues of grafts stored at about 18° C. for two days. Activity first becomes evident in tissues of the bark, as shown by mitotic figures (fig. 1), by the general expansion of this region, and by the presence of linear series of cells which have resulted from recent cell divisions (fig. 2). Although the preparation of the tissues was not designed to yield cytological details, the numerous minute chromosomes were readily discerned in metaphase plates. The orientation of the mitotic figures is variable, but the spindle tends to be at right angles to the cut surface, and several successive divisions commonly occur in one plane (figs. 2, 3). If the cut surface is clean, meristematic activity begins in cells on the very surface of the cut (fig. 2). If the surface cells are injured, activity is more likely to begin deeper within the tissues (fig. 3).

Some preparations show early activity in the cambium, and the cambium and the most recently formed secondary phloem may produce most of the callus (figs. 11, 12, 13). However, it has become more and more evident during this study that the cambium may be a minor factor in the formation of callus. The primary cortex and the older secondary phloem, located well outside of the cambium, may in some cases produce most of the callus. In fact, callus may be derived largely from primary cortex of the scion (figs. 5, 12) or from secondary phloem of the stock or scion (figs. 12, 14). The tissues of the bark may contribute variable proportions of the total callus. In this material the periderm does not seem to produce callus. This is particularly evident in figures 4, 6, 12, 31. The extent

of the proliferating area is variable. Only limited areas may produce callus (figs. 10, 11, 12, 13) or nearly the entire cut face of the bark undergoes proliferation (figs. 4, 14, 31).

In the manufacture of grafts by the usual nursery methods, the grafts are frequently subject to conditions which interfere with callusing and healing. If the cut surface of the bark is kept clean and if excessive desiccation does not occur, extensive superficial callusing takes place as just described. But in most of the preparations of early stages examined, there were noted more or less extensive dark areas of dead cells (figs. 4, 11, 12, etc.). These cells were found to be crushed, and grains of sand were abundant on the surface. It is probable that the dead areas were the result of crushing, injury by grit, or drying, somewhere along the process of making and storing the grafts. Regardless of the cause of the formation of these inactive areas, they constitute a barrier to effective contact between the stock and scion. The duration of the barrier action is variable. Active proliferation of the deeper tissues may eventually rupture the dead surface layer (figs. 4, 6, 10, 11). Subsequent coalescence of the stock and scion leaves more or less displaced sheets of dead tissue buried in the callus (figs. 5, 7, 14, 28). In some cases, extensive and more permanent inactive areas seem to retard callusing of the member involved (figs. 11, 12, 13, 15).

The foregoing description of the early stages of callusing takes into account various forms of mechanical damage to the meristematic surface. Since the grafts were not made under aseptic conditions, it is not at all surprising to find that, under conditions of excessive humidity and temperature, fungi gain a foothold on the cut surfaces. The disorganized area in the bark of the scion in figure 9 contains abundant septate mycelium, strands of which extend across the gap to the root. The effect of this invasion and destruction of the tissues obviously prevents proliferation and retards or prevents union in this region between members of the graft.

In clean, well-made grafts, in which the stock and scion are reasonably well matched, unobstructed contact is soon made between the respective calli of the members. The spongy callus cells mingle, and growth pressure compresses the mixed callus until the respective cells of the stock and scion become indistinguishable (figs. 4, 5, 14).

No attempt has been made in the present study to accomplish differential staining of stock and scion cells. After contact is established, the cells on the outer tangential surface of the callus become suberized (figs. 4, 5). Outward radial proliferation is thus effectively stopped, and cell division then becomes confined to the limited exposed surface in the space between members of the graft (figs. 4, 5, 14, etc.). Proliferation continues on this free surface; and in very rare cases, especially near the scion lip and the "shoulder" of the root, the gap between stock and scion may be filled by parenchymatous "filler callus" by the end of the first growing season. In most grafts, however, especially in the median region of the graft union, the space between the members is not filled during the first season.

In the varieties of apple used in this study, no proliferation occurs during the first growing season from the xylem rays, the cut ends of which terminate in the gap between the members (figs. 10, 20, 21, etc.). These wood ray cells, though living, do not undergo division. The other xylem elements and the pith produce no callus whatever. Examination, under higher magnification, of the contact between the filler callus and the contiguous wood ray cells shows clearly the thin-walled spongy callus cells pressed into the cut lumina of the thick-walled xylem ray cells. The filler callus between the woody cylinders, as well as the rest of the callus of the graft, is derived entirely from tissues of the bark.

Cambial bridging between stock and scion

If the stock and scion are radially matched along a particular edge of the cut the respective cambia are practically on the same arc, and the tangential gap may be filled by callus in less than two weeks. With a larger gap a longer period may be required (fig. 12); but in any reasonably well-matched and clean gap, unobstructed mingling occurs between the spongy, undifferentiated callus cells derived from the stock and scion respectively (figs. 4, 5, 14). Although the cells derived from the stock and scion soon become indistinguishable, it is evident from the early stages that the stock and scion do not necessarily contribute equal quantities of callus. In fact, most of the callus in the gap may have been produced by one or the other member.

After the gap between the cambia becomes filled with callus, the formation of a "bridging cambium" begins. Along the cut edges of the old cambium, the adjacent callus cells undergo several divisions radially, producing several layers of narrow, tangentially elongated cambium-like cells. This activity extends tangentially to callus cells farther away from the respective cambia of the stock and scion. The callus between the members is finally "bridged" by a distinct, cambium-like layer of deeply staining cells with large nuclei (figs. 7, 8). In view of the structure and subsequent activity of these cells, they constitute a true cambial region. The important features of the development of this new cambium are that the cells differentiate from spongy callus, that re-activation begins adjacent to the old cambium, and that differentiation progresses tangentially until a cambial arc connects the respective cambia of the stock and scion. The period required to achieve this bridging is very variable, but many good cases of successful cambial union were noted in grafts three weeks old.

Effect of accurate fitting of the graft on the healing process

Perhaps the most important factor in producing successful grafts is the fitting of the joint between the stock and scion. Although it is generally known that a properly made graft is more likely to make a good tree than is a poor union, the exact histological details of the failure of healing have not been clearly understood. In order to emphasize the desirability of making good grafts, the comparative histology of good and poor unions has received particular attention in this study.

The most common type of misfit in piece-root tongue grafts is the result of combining a small scion and a large root. The root is often 50 per cent larger than the scion. Occasionally the scion, regardless of its relative size, is so placed that its edge projects over the edge of the root. If the cambium of one member of the graft falls outside of the cambium but within the periderm of the other member, the respective calli can make contact, the extent and effectiveness of the contact depending on the degree of overlapping (figs. 10, 14, 16). The mixed callus, derived from both members, as shown in figures 10 and 14, proliferates inward as well as outward. On the outside

margin of the cleft the exposed callus proliferates rapidly, at first by the division of surface cells, and later by the division of cells below the surface, building up ridges and irregular masses of callus. In figure 16 the entire cut surface of the root seems to have been inactive, and the entire callus was produced by the scion. In this case the new cambium differentiated from the callus, instead of continuing the cambial arc, tends to be curved inward (fig. 16 *b*) or outward, and obviously cambial bridging between the stock and scion is delayed or even prevented.

If the cambium of one member falls radially outside of the periderm of the other member, an exposed callus proliferates from the active tissues of the protruding member (figs. 11, 13, 15, etc.). The free callus presently comes into contact mainly with the cork of the recessed member. Such contact is highly ineffective from the standpoint of establishing a successful graft union. In the graft shown in figure 12, the periderm and cortex of the recessed scion had been cut away. The exposed tissues of the scion had proliferated outward, while the cambium and secondary phloem of the root proliferated inward. Contact between the calli may often take place in this way.

The foregoing description of cambial bridging has taken no account of secondary activity in the old cambium of the graft. Since callusing follows the breaking of dormancy, a corresponding reactivation of the dormant old cambium is to be expected during the period of callusing. The cells of the old cambium begin to divide several days after the grafts are made, and in ten days an appreciable increment of xylem is laid down (fig. 6). This cambial activity seems to aid in rupturing any barrier layer of dead tissue (fig. 14 *a*) in the vicinity of the cambium, and probably facilitates early cambial bridging.

Progress of healing after cambial bridging

During the three weeks' interval just described, the behavior of the callus along the margin of the cleft and in the gap between the members is variable, depending on the matching of the edges. The inward proliferation of filler callus progresses by the division of the spongy cells on the inner surface of the callus (figs. 4, 14). The cells of the callus between the xylem cylinders of the members do not differentiate into vascular elements during the first season. Inward growth of the callus is soon terminated by the formation of a suber-

ized layer on the inner surface. Figure 18 shows this limiting layer to be well developed in twelve weeks. The remaining gap probably does not become subsequently filled with callus.

The development of the marginal callus along the outer edges of the union is much more influenced by the matching of the members than is the history of the filler callus. On a well-matched edge very little callus is formed beyond the edge of the cleft. The surface cells become suberized and further outward proliferation is effectively stopped (figs. 5, 7, 27 *a*). In a poorly-matched graft there is known to be a tendency to produce excess callus (figs. 10, 11, 13, 15, 27 *b*, etc.). This condition is associated with delayed cambial bridging. At first the free marginal callus consists of undifferentiated parenchyma. Presently there appears a concentric zonation of the cells, until a well-defined cambium-like zone develops (fig. 13). Centrifugal expansion of the excess marginal callus progresses by tangential cell divisions in this meristematic zone. In the older central regions of the callus, some of the cells differentiate into contorted xylem elements. Further work is now under way on the histology of the continued growth, internal differentiation, and ultimate fate of excess callus.

In a well-matched graft, the establishing of transverse and vertical cambial continuity produces a complete layer of cambium over the union. As previously described, typical cambial activity begins in the newly differentiated cambium as well as in the old cambium. It has long been known that after this point, continued normal activity of the cambial sheath adds successive increments to the vascular cylinder during the life of the tree. Transverse sections through representative "good" trees (over 3 feet high) during the first season show good vascular continuity on all or most of the six planes of contact (figs. 19, 27). This condition is described in more detail in the legends of the illustrations and hence requires no extended description here.

The histological details of the course of development have been less clearly understood in the case of poor unions than in good unions. As shown in figure 16, a recurved cambial extension is differentiated from the callus in a poorly-matched graft. This may occur even in a fairly good match (fig. 17). Typical cambial activity takes place in

this new cambium as well as in the original cambium of the member. The result of this activity is the laying down by the old cambium of a normal xylem increment to the original xylem cylinder, while the new, incurved or outcurved cambium lays down a partial cylinder or even an irregular bar of xylem on the margin of the member (figs. 20, 21, 22). Thus there is no continuity between the respective xylem increments of the stock and scion, the only contact being between the proliferated calli of the adjacent members. In some grafts, the parenchymatous area between the members is eventually bridged by cambium differentiated from the callus, and a continuous vascular cylinder is subsequently laid down.

Summing up briefly the comparative histology of successful and unsuccessful unions, it seems that the relative size and vigor of the grafted tree by the end of its first growing season are largely conditioned by the continuity of the second annual ring of the tree, laid down during the current growing season. Changes occurring in the first annual ring (the original stem and root used in making the grafts) probably have no influence on the development of a successful union.

Formation of callus knots on the scion lip

One of the principal objectives of this study has been to trace the origin and development of the gall-like knots which occur on the scion lip. The orientation of the scion lip with respect to the adjacent root is highly variable in the general run of grafts. At one extreme the oblique face of the short scion lip does not cover the cut on the root. Consequently the cut tissues of the bark of the scion are in contact with the wood of the root, while the cut bark tissues of the root are exposed. At the other extreme the long scion lip extends beyond the corresponding face of the root; hence the cut bark of the root may be in contact with scion wood, and there is an exposed area on the under side of the scion lip. Between these extremes all intermediate conditions are found.

Since it is impossible to study the anatomy of a given underground knot through the season, collections for this study were made at intervals during the season, selecting representative "good" trees

over 3 feet high, and "poor" trees less than 1 foot high. Obviously the presence or absence of overgrowths was not known before digging. There was found to be much variation in size and degree of differentiation in overgrowths of a given collection. In the present paper, emphasis is placed on the comparative histological relationships in the union in thrifty and in poor trees, and in knot-free and knotty trees.

A radial section through the scion lip ten days after grafting shows clearly that any or all living tissues of the bark, excepting the periderm, may produce callus (fig. 31). The progress of the wound reaction and the tissues involved are the same in the case of the scion lip as in other parts of the union. In view of these similarities, the matching of the scion lip may be expected to bear some relation to excessive callusing on the scion lip.

Figure 32 shows a representative case in which a knot had developed on a "short" scion lip, in which the tip of the scion lip was in contact with the wood of a relatively large root. Obviously the callus of the scion lip was not in contact with root callus, and as in the cases of lateral misfits, excessive callusing occurred on the scion lip. The knot illustrated had developed in three weeks in storage. The large knot shown in figure 35 developed on a long, overlapping scion lip. Since the active tissues of the scion lip were not in contact with callusing tissues of the stock, coalescence of respective calli did not occur, and excessive callusing of the free scion lip formed a knot. Some vascularization of the knot tissues had taken place, as shown by the irregular zone of xylem (*d*).

It is generally believed that the lip knot consists entirely of scion tissue, and this is probably true in many cases. The present work, however, has shown that as in the formation of excess marginal callus, both stock and scion may contribute to the callus of the lip knot. The bark on the cut faces of the root produces some callus, and if this callus comes into unobstructed contact with the callus of the scion lip, some degree of coalescence occurs. Since the rate of callusing is greater on the free member, the scion, the bulk of the knot is scion callus. But as shown in figure 34, more or less stock (root) callus may become incorporated into the knot. From the

standpoint of the possible origin of bud primordia in the callus, it should be borne in mind that the callus may consist of both stock and scion tissue.

Knots are known to occur on the scion lip in large, well developed apple trees, although this is contrary to the usual rule. In the absence of isolation data it is not improbable that in these field-grown trees some of the older knots may be crown gall, but histological study shows that the knot on the scion lip of a vigorous tree is usually associated with poor healing of the lip joint, whereas the rest of the union is properly healed. In this case the tree as a whole may become vigorous, but a large knot may nevertheless develop on the scion lip.

This work has shown that local regions of the graft union may react more or less independently in the development of excess callus on various parts of the union. The vigor of the grafted tree and the formation of excess callus are both determined, though independently, by the vascular effectiveness of the tissues laid down incident to healing. Non-pathogenic overgrowths are masses of excess callus, and their development is determined by the conditions which influence the phenomena of callusing.

Discussion

Of the various possible stimuli which may initiate the processes of repair, the most obvious are external mechanical forces. SMITH (13), however, has shown that proliferation can be induced in the pith of *Ricinus* by the use of chemicals, entirely without mechanical means. It seems that mechanical forces are not necessarily the exclusive stimulatory agencies, and we may look for some factor within the tissues.

A possible internal agency may be sought in the food relationships in the organs involved. KÜSTER (6) and others have stated that organs containing abundant food, callus more extensively than organs having little food. In stored and comparatively inactive apple grafts, however, callus knots are generally more prominent on the scion, in which there is relatively less food, than on the root, which is literally full of starch. This is not in harmony with KÜSTER'S observations. Perhaps callusing is influenced by the alteration of

stored food following dormancy rather than by the quantity of food present.

Photosynthesis can certainly be eliminated as a factor during the early stages of callus-knot formation prior to planting in the field. However, RIKER and KEITT (12) and MUNCIE (8) have shown that callus knots are associated with poor graft unions on growing trees; and it seems probable from the present histological study that, during the growing season, lack of vascular continuity brings about the accumulation of the products of photosynthesis and favors the continued enlargement of callus knots.

The rôle of the cambium seems to be somewhat over-emphasized in the literature, particularly in textbooks.² BRADFORD and SITTON (2) state that pith cells and xylem ray cells (of apple?) produce callus. These writers did not state the source of this information and it is not clear whether they refer to matured ray cells or to recent cambial derivatives. Figures by BAILEY (1) and COE (3) show that in bud grafts, which are made at a time of rapid cambial activity, the partly differentiated cells of the new xylem proliferate to contribute some of the wound callus. The present work does not support FISK's (4) statement that wood rays and wood parenchyma produce wound callus.³ At least in the varieties of apple studied, wound callus is produced only by tissues of the bark, that is, the region outside of the xylem cylinder.

Although the necessity of vascular continuity between the members of a graft is clearly understood, there seems to be an impression among nurserymen and others interested in grafting that the cavity (fig. 24, etc.) between the members is responsible for poor conduction, and that the filling in and vascularization of this cavity would improve conduction. MUNCIE and BERKHOUT (7) have found a significant reduction in the rate of flow of water through defective

² HOLMAN and ROBBINS, *Textbook of general botany*. 1924: "... the cambium is stimulated to active growth and forms a mass of large, thin-walled cells known as callus." EAMES and MCDANIELS, *An introduction to plant anatomy*. 1925: "Callus may be formed by the division of parenchyma cells in the phloem and cortex, but its most frequent source is the cambium."

³ In view of the fact that several investigations dealing with the histology of the graft union have been made in the Botany Department of Iowa State College, the unpublished manuscripts, available at the library of this college, are cited in this paper.

unions. The cause of this poor flow is not the failure of vascularization between the original xylem cylinders of the members, but the failure to lay down an effective vascular ring during the first growing season of the union. In connection with studies on compatibility, PROEBSTING (10, 11) has shown that in numerous incompatible interspecific grafts in *Prunus* and *Pyrus* the new tissues between the members are parenchymatous. PROEBSTING attributes unsuccessful grafts to failure to form vascular tissues connecting the members, but he does not state clearly what he means by "vascularization." The present data concerning the process of "cambial bridging" and the subsequent laying down of a vascular sheath are applicable to the morphological aspects of compatibility.

Associated with the general problem of continuity between the members are such problems as protoplasmic continuity, and the matching of pits between adjacent cells of stock and scion respectively. It seems that some workers have not distinguished clearly between plasmodesma and pits. Illustrations by COE (3) and BAILEY (1), represented as being plasmodesma, undoubtedly represent simple pits and highly reduced full-bordered pits. An adequate technique for approaching these problems has not yet been developed.

The present study has a direct bearing on the crown gall problem. Since the grafts used were not handled under aseptic conditions, the presence of various organisms must be accepted. Furthermore, after grafts are planted in the field they are subject to contact with the soil flora, and the subsequent entry of organisms into the tissues is not precluded. But from this study of the progress of callusing taking place in sterilized peat, from the earliest mitoses in the graft to the formation of large knots, it is probable that at least the early stages of callusing, as here described, represent the condition in non-bacterial overgrowths. These non-pathogenic overgrowths are overdevelopments of the callusing incident to healing, brought about by some disturbance in the healing process.

Comparing the histological development of these non-pathogenic overgrowths with the histology of crown gall, as described in the literature, largely on plants other than apple, shows several basic similarities in the development of the two types of overgrowth.

Further anatomical comparison of overgrowths, shown by isolation studies to be either bacterial galls or non-pathogenic callus knots, may reveal reliable contrasting criteria for distinguishing between the two types of overgrowth.

Summary

1. The histology of the healing of apple grafts during the first growing season is described, with special emphasis on the formation of overgrowths known as callus knots. Piece-root tongue grafts of Wealthy scion on French crab root were used.
2. Callus is produced exclusively by tissues located outside of the xylem cylinder. Any living tissue of the bark, excluding the periderm, may proliferate. The cambium may contribute very little of the callus.
3. Proliferation is inhibited locally by crushing of the surface cells, by desiccation, or by destruction of the tissues by fungi.
4. The gap between the respective xylem cylinders of the stock and scion does not become filled with callus during the first season. Indications are that the gap does not become filled during subsequent years. The limited amount of parenchymatous filler callus formed does not become differentiated into vascular tissues during the first year.
5. Unobstructed contact between the respective calli of the stock and scion favors a mingling of the parenchymatous cells, and the continued proliferation of a mixed callus. No method has been found for distinguishing microscopically between stock and scion cells in the mixed callus.
6. In well-matched grafts, an arc of cambium, continuous with the respective cambia of the stock and scion, is differentiated from cells of the intervening callus. The complete cambial layer sheathing the union subsequently lays down a vascular cylinder, which comprises the second annual ring of the tree.
7. The size and vigor of the tree at the end of the first growing season are conditioned by the degree of effective continuity in the new vascular sheath around the union.
8. Excess callus is proliferated wherever there is a local obstruction to effective vascular union between the stock and scion. Large

masses of callus, known as callus knots, develop on the scion lip as the result of obstructed union between the scion lip and the stock.

9. Localized callusing may be independent of the general vigor of the tree. Histological interpretations are suggested for the presence of large lip knots on good trees, and the absence of pronounced knots on poor trees.

10. The size and permanence of lip knots or other masses of excess callus are conditioned by the extent and permanence of obstructions to vascular union.

11. Certain phenomena that have been attributed to incompatibility may be interpreted as the result of poor grafting.

12. The internal differentiation of masses of non-pathogenic excess callus resembles somewhat the previously described histology of crown gall.

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EXPLANATION OF PLATES IV-VII

PLATE IV

FIGS. 1-8.—Fig. 1, mitotic telophase in primary cortex of scion lip. Regions of graft from which sections were taken are indicated in the sketches. $\times 500$. Fig. 2, stock (root) of graft 2 days after grafts were put into warm storage. Indications of recent cell divisions at *a*; expansion of cells in area *b* prior to division. $\times 45$. Fig. 3, pericycle and primary cortex of scion; surface layer of dead cells (*a*) through which the spongy callus cells (*b*) have proliferated. Meristematic, cambium-like layer has differentiated parallel to surface. Graft 10 days old. $\times 100$. Fig. 4, well-matched edge of a graft 3 weeks old. Scion callus derived from entire cut face of bark, excepting periderm. Dead surface layer (*a*) which had covered original cut face of the member pushed out by callus. Root callus produced by secondary phloem and cambium. Small mass of callus (*b*) has pushed through the obstructing layer (*c*). $\times 22$. Fig. 5, matched graft 3 weeks old. Inactive surface layer (*a*) limits proliferation. Mixed callus (*b*) is the beginning of "filler callus" between the xylem cylinders of the members. $\times 28$. Fig. 6, edge of graft 10 days old (note almost complete absence of callus along plane of contact). Appreciable increment of xylem and phloem had already been laid down in 10 days. Cambia of members not yet connected. $\times 28$. Fig. 7, cambial bridging in well-matched graft 3 weeks old: *a*, inactive surface layer on original cut face; *b*, filler callus derived largely from scion; *c*, dead tissue limiting outward callusing. Note deeply stained layer of bridging cambium. $\times 18$. Fig. 8, enlarged section of cambial bridge. $\times 100$.

PLATE V

FIGS. 9-18.—Fig. 9, moldy graft 3 weeks old: *a*, disorganized area producing no callus; *b*, strands of septate mycelium. $\times 28$. Fig. 10, graft 10 days old: *a*, dead layer ruptured by scion callus (*b*); *c*, filler callus produced entirely by cambium and secondary phloem of stock. $\times 22$. Fig. 11, poorly-matched graft 3 weeks old. Extensive dead area (*a*) on stock. Excess marginal callus (*b*) produced where root callus did not coalesce with scion callus. $\times 22$. Fig. 12, scion callus derived from primary cortex which may eventually coalesce with root callus. Graft 3 weeks old. $\times 18$. Fig. 13, some coalescence of stock and scion calli occurring in graft 3 weeks old. Internal differentiation of excess callus begun. $\times 12$. Fig. 14, graft 3 weeks old, showing displacement of strip of dead barrier tissue (*a*) which had been continuous with (*b*). Filler callus (*c*) is a mixed callus derived from both the stock and scion. $\times 22$. Fig. 15, excess callus produced on margin of stock in 3 weeks. $\times 20$. Fig. 16, a poor graft 3 weeks old. Callus produced by scion only. Excess callus (*a*) derived from primary cortex; filler callus (*c*) produced by cambium. Recurved cambial layer (*b*) has differentiated in the callus. $\times 18$. Fig. 17, recurved, new cambium in stock callus and in mixed callus (note absence of proliferation from pith of scion, *a*). Graft 3 weeks old. $\times 18$. Fig. 18, graft dug after

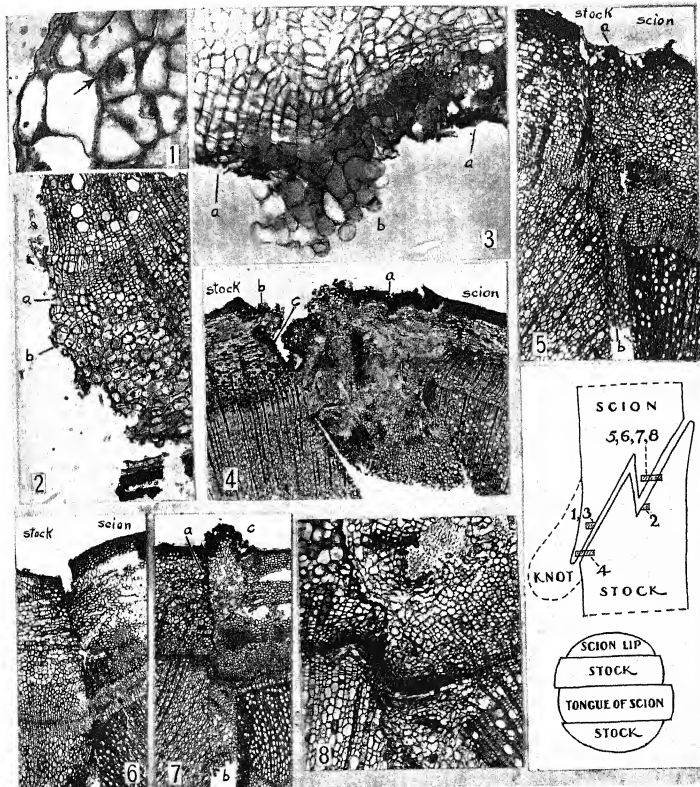
12 weeks in the field (note rolled-in ridge of xylem on edge of each member, produced by activity of recurved cambium shown in fig. 17). Present cambium is nearly straight. Suberized layer (*a*) limits inward proliferation of filler callus. $\times 12$.

PLATE VI

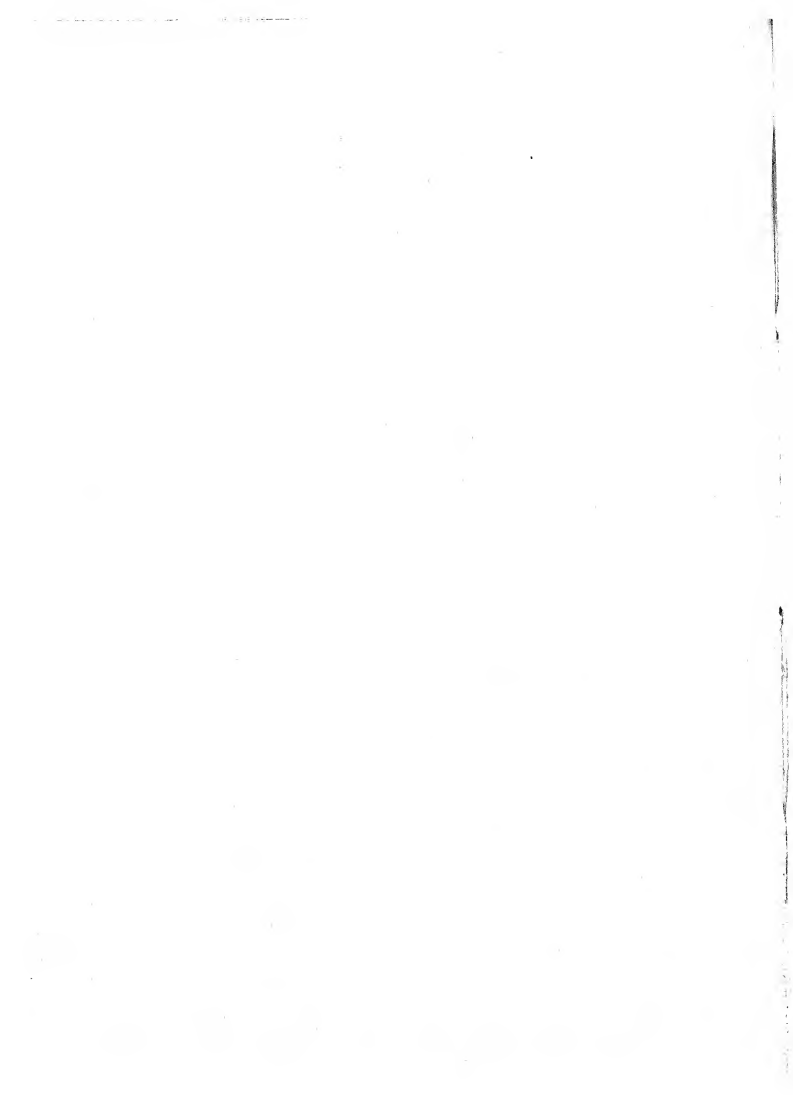
FIGS. 19-27.—Fig. 19, tree 3 ft. high, having a small lip knot, dug after 12 weeks in field. Xylem cylinder of the current season is practically continuous. Callus does not completely fill cavity between original members of graft. $\times 5$. Fig. 20, very poor tree, dormant. Scion cleft has healed on sides *a* and *b*, and partly at *c*. On side *d* a bar of vascular tissue has been laid down by the recurved cambium. Cf. narrow annual ring with fig. 19. $\times 5$. Fig. 21, small knotty tree, showing almost completely separated scion lip. On side *a*, vascular continuity is much better than on side *b*. $\times 5$. Fig. 22, small dormant tree. Marginal layers of vascular tissue *a* and *b* are not in continuity with adjacent member. Root cleft has healed over at *c*. $\times 5$. Fig. 23, very small tree (less than 1 ft. high) having a large lip knot. Side *a* is fairly well healed; on side *b* there is no vascular continuity; on side *c*, continuity is afforded only through parenchymatous tissues. $\times 5$. Fig. 24, small dormant tree. Owing to defective matching, cambial continuity was established late in season. $\times 5$. Fig. 25, very poor, knotty tree at end of first season. Note almost complete lack of vascular continuity throughout the union. $\times 4$. Fig. 26, small knotty tree, having a lip knot on side *a*. Other planes of contact also poorly healed. Negligible amount of callus (*b*) proliferated from cut end of a broad parenchymatous ray of root (this condition is unusual). $\times 6$. Fig. 27, good tree in which the new annual ring is fairly continuous on side *a* and on part of side *b*. Owing to a poor fit on one edge of side *b*, cambial bridging did not occur and a large mass of excess callus had developed. $\times 5$.

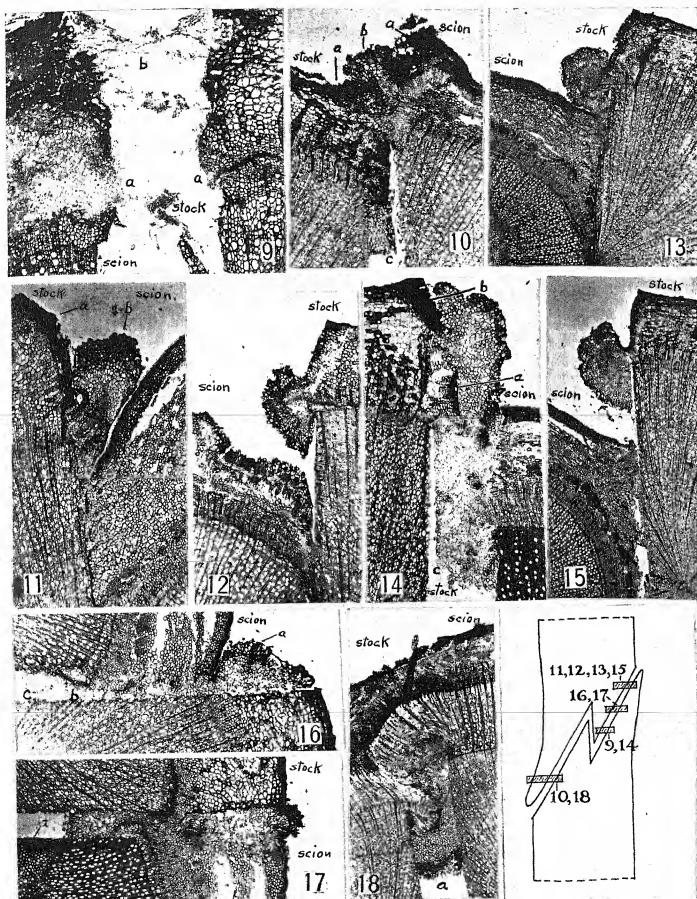
PLATE VII

FIGS. 28-36.—Fig. 28, poor tree in which the scion lip is partly separated from the root by layer of dead tissue and a gap at *a*. Members are connected only by parenchyma (*b*). $\times 6$. Fig. 29, mass of callus at *a* was produced mainly by secondary phloem of stock; callus *b* was produced by cambium of the stock. Pressure on the uncut bark of stock has induced proliferation in primary cortex (*c*). $\times 20$. Fig. 30, edge of scion lip on graft 5 weeks old. Scion has produced a mass of callus (*a*), which had proliferated inward at *b*. Effective contact prevented by the area of dead tissue. Small knot present below this level. $\times 12$. Fig. 31, callus knot developed on scion lip in 3 weeks. No proliferation had occurred in the periderm *a* and the wood *b*. $\times 18$. Fig. 32, lip knot 3 weeks old. Callus (*a*) was formed by the proliferation inward of bark callus. In area *b*, some differentiation of callus into contorted xylem elements had taken place. $\times 12$. Fig. 33, scion lip knot from tree 3 ft. high, dug in August. Region of callus (*a*) derived from the stock has coalesced with scion tissue (*b*) of knot. $\times 4$. Fig. 34, lip knot 12 weeks old. Layer of xylem (*a*) is stock tissue; area (*b*) of the knot is stock (root) tissue. $\times 4$. Fig. 35, lip knot 3 weeks old. Tissue *a* was derived from secondary phloem (*b*) of root. Coalescence with the scion knot may readily occur. Note absence of proliferation on the wood (*c*). At *d*, the knot tissues have differentiated into xylem. $\times 3$. Fig. 36, detail of tangential section at margin of lip knot. Enlargement is brought about by activity of layers and whorls of meristematic tissue. Centers of the older whorls differentiate into contorted xylem elements. $\times 45$.

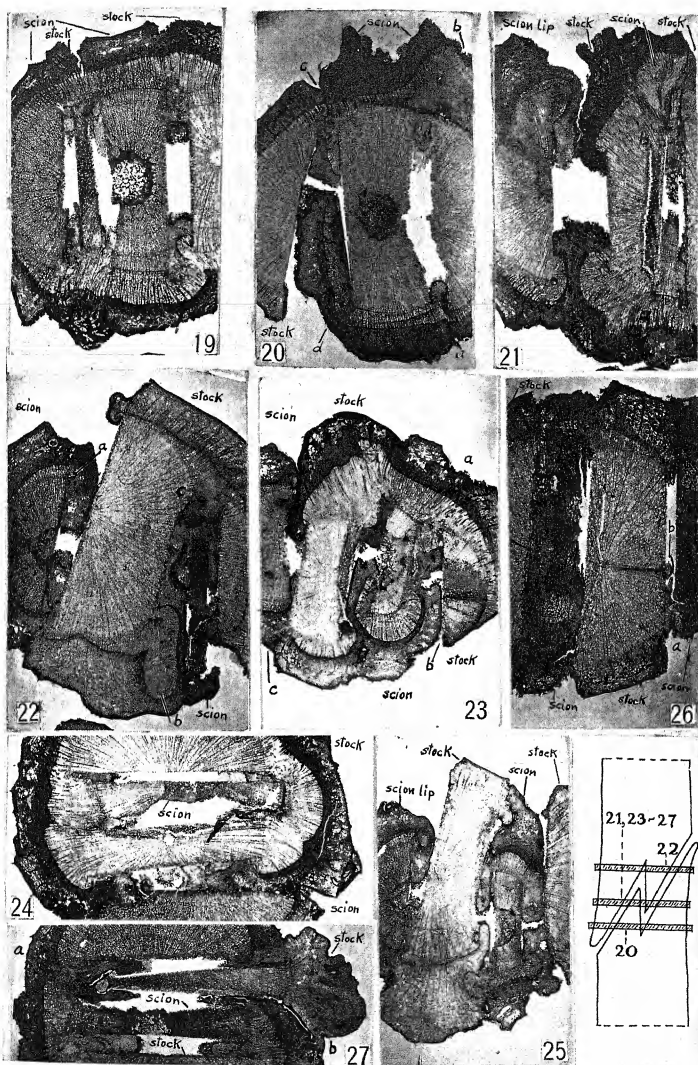


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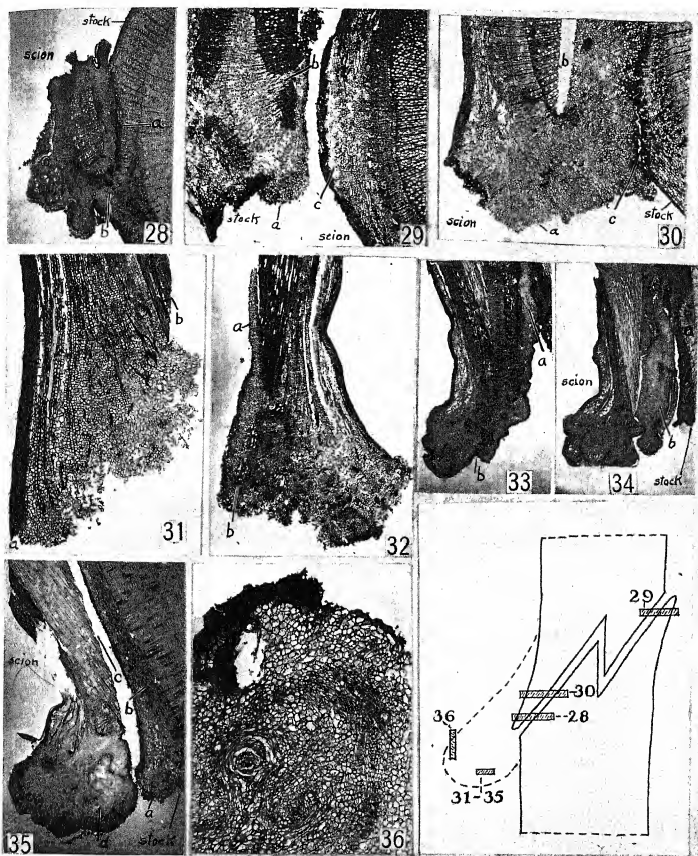




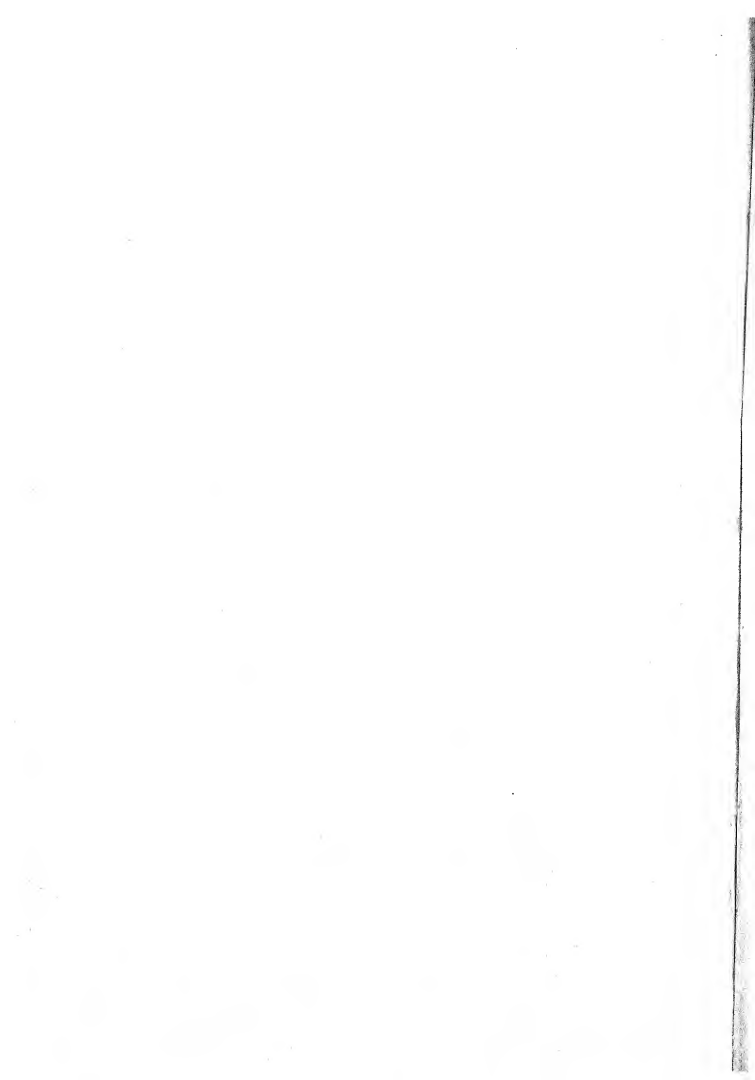
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MINERAL AND NITROGEN CONTENT OF THE LEAVES OF SOME FOREST TREES AT DIFFERENT TIMES IN THE GROWING SEASON¹

J. S. MCHARGUE AND W. R. ROY

Introduction

The mineral and nitrogenous residue which resulted from the annual deposition and decay of forest leaves during prehistoric times was the principal source of the fertility of virgin soils. After the arrival of European settlers in this country magnificent forests began to be cleared away, and the reserve fertility which had been accumulating from decaying leaves and trees through the past ages began to be drawn upon in the processes of leaching, erosion, and the growing of farm crops. During the 300 years in which the art of agriculture has been practiced in this country, considerable areas of land, within the humid regions, have been abandoned for purposes of cultivation because the soils have been so depleted of their virgin fertility that it is no longer possible to produce farm crops upon them. In recent years there has been considerable discussion concerning the reclamation of abandoned farm lands through the process of reforestation.

The growth of important forest trees to a productive state is a slow process under the most favorable conditions. Accordingly, any information that contributes to a more adequate understanding of the kind and amount of the mineral nutrients necessary for the most rapid growth of forest trees may be of some practical and economic importance in the process of reforestation and the conservation of abandoned farm lands.

SEREX (5) analyzed leaves collected in the spring and autumn from certain important species of forest trees, and from the results

¹ Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station.

The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

of his analyses arrived at the conclusion that the average fertilizer value of a ton of forest leaves was approximately \$5. TSCHERMAK (6) studied the agricultural value of forest litter and concluded that the absorptive power of the litter increases rapidly with the degree of decomposition, and that its fertilizing value is due to the nitrogen and mineral matter contained in the annual crop of forest leaves.

SEIDEN (4), in a study of the external factors that affect the ash content of plants, found that the ash content of the leaves of trees decreases from the bottom toward the top. He emphasizes the importance of taking leaves from the same part of the plant for comparative analyses. He concludes that the ash content of leaves tends to vary with the amount of water transpired, and that, in the fall, plant nutrients tend to migrate back into the branches. QUARTAROLI (3) reports that the manganese content of leaves increases with the age of the leaf, whereas the copper content decreases. Furthermore, he suggests that copper is concerned in plant metabolism, possibly in a way similar to the vitamins in animal metabolism.

For some time the senior writer and co-workers have been interested in the occurrence, distribution, and probable function of some of the less common elements in plants and animals. In previous reports (2) it has been shown that manganese, copper, zinc, and boron have important functions in the metabolism of plants and animals. This paper reports further data on the occurrence of manganese, copper, and zinc in the leaves of 23 species of trees, and also the percentages of ash, silica, iron, calcium, magnesium, phosphorus, potassium, sodium, sulphur, nitrogen, fat, and crude fiber. The leaves were collected at three different times during the growing season of 1931, from trees growing near the Kentucky Agricultural Experiment Station, in soil derived from a limestone formation. The first set of samples was collected May 4th to 8th, when the leaves were about half grown; the second, August 3rd to 6th, about mid-season; and the third, September 4th to 6th, a short time before frost. About three pounds of normal green leaves were gathered from different places and heights on the same trees each time. The leaves of the elm, black oak, and black walnut were somewhat smaller than half-grown when the first samples were taken. The mulberry tree was sampled only in September.

Experimental work

After weighing, the fresh leaves were thoroughly air-dried at room temperature, re-weighed, ground, and put into air-tight containers for analysis. One hundred grams were weighed into silica dishes and heated over the low flame of a Bunsen burner until the easily volatile matter was expelled, after which the dishes containing the partly ashed residues were transferred to an electric muffle furnace and the ashing was finished at about 600° C. The dishes containing the ashes were allowed to cool in desiccators and weighed. The ash was moistened with distilled water, the dish covered with a watch glass, and 1-1 hydrochloric acid run under the watch glass through the lip of the dish until the carbonates were decomposed and 5 ml. excess of the acid had been added. The watch glass was rinsed into the dish, the latter transferred to a hot-water bath, and the contents brought to dryness, after which the residue was baked on a sand bath until all the odor of hydrochloric acid was expelled. The dish was allowed to cool, the residue moistened with 5 ml. of 1-1 hydrochloric acid and about 25 ml. of hot water, and digested on the hot-water bath. An excess of hydrochloric acid at this point is to be avoided because too much acid will interfere with the precipitation of the small amount of copper present; therefore just enough dilute 1-1 hydrochloric acid to bring the acid-soluble salts into solution is all that is necessary. The silica was filtered on an ashless filter paper, thoroughly washed with hot distilled water, dried, ignited, and weighed. The filtrate was transferred to a 150 ml. Erlenmeyer flask and made to a volume of about 100 ml., heated to near the boiling point, and a slow stream of hydrogen sulphide gas bubbled through the solution for about 15 minutes while the flask was rotated, after which the flask was stoppered tightly and set aside for several hours, preferably over night or until the dark-colored precipitate had settled completely and the supernatant solution was clear. The precipitate of copper sulphide was filtered on a pad of paper pulp held in a Caldwell crucible, and washed with a dilute solution of hydrochloric acid which was saturated with hydrogen sulphide, transferred to a porcelain crucible and ignited to the oxide, cooled, dissolved in a few drops of nitric acid, and copper determined colorimetrically by the Xanthate method. The filtrate

TABLE I

ANALYSES OF LEAVES OF 23 SPECIES OF TREES COLLECTED AT THREE INTERVALS DURING GROWING SEASON;
RESULTS CALCULATED AS PERCENTAGES OF MOISTURE-FREE MATERIAL

SPECIES	SAM- PLE NO.	AN- TIM- ON (%)	IRON (%)	MAN- GAN- ESE (%)	ZINC (%)	CAL- CIUM (%)	MAG- NESIUM (%)	PHOS- PHORUS (%)	POSS- SIUM (%)	SODI- UM (%)	SUL- PHUR (%)	NITRO- GEN (%)	PRO- TEIN (%)	FAT (%)
<i>Pyrus coronaria</i> (crabapple)	1	5.87	0.200	0.0080	0.180	0.020	0.007	1.27	0.23	0.34	1.79	0.10	13.5	14.9
	2	6.54	0.340	0.0080	0.310	0.020	0.032	1.92	0.32	0.39	1.42	0.21	9.4	10.3
	3	7.92	0.370	0.0090	0.030	0.030	0.028	1.84	0.35	0.50	1.54	0.16	7.6	12.0
<i>Fraxinus quadrangulata</i> (ash)	1	6.51	0.340	0.0060	0.280	0.060	0.012	0.63	0.43	0.61	2.04	0.40	3.8	5.45
	2	7.12	0.880	0.0110	0.330	0.070	0.032	1.01	0.34	0.35	1.32	0.05	3.09	2.46
	3	8.43	1.090	0.0130	0.250	0.080	0.034	1.98	0.30	0.42	1.26	0.29	0.11	2.10
<i>Aesculus glabra</i> (buckeye)	1	8.74	0.340	0.0050	0.430	0.070	0.035	1.57	0.46	0.61	2.55	0.32	4.11	25.7
	2	10.27	1.470	0.0070	0.950	0.100	0.038	3.66	0.43	0.28	1.19	0.05	2.08	13.0
	3	14.44	1.730	0.0070	0.940	0.120	0.038	4.37	0.31	0.29	0.92	0.11	1.60	10.0
<i>Catalpa speciosa</i> (catalpa)	1	8.15	0.380	0.0080	0.330	0.080	0.034	1.17	0.48	0.58	2.47	0.16	2.3	3.68
	2	7.30	0.840	0.0210	0.950	0.100	0.038	1.85	0.31	0.31	1.45	0.12	2.22	2.47
	3	9.62	0.800	0.0190	0.680	0.130	0.030	2.26	0.34	0.30	1.31	0.10	0.48	1.94
<i>Prunus serotina</i> (cherry, wild black)	1	6.62	0.100	0.0050	0.210	0.170	0.031	1.29	0.37	0.47	1.31	0.14	0.21	3.55
	2	8.45	0.160	0.0080	0.210	0.260	0.072	2.37	0.55	0.14	1.14	0.06	2.63	10.4
	3	8.04	0.300	0.0080	0.290	0.230	0.027	2.20	0.53	0.35	1.09	0.07	1.24	14.0
<i>Magnolia macrophylla</i> (cucumber tree)	1	8.16	0.160	0.0080	0.150	0.030	0.039	0.90	0.39	0.48	3.30	0.19	0.29	4.31
	2	7.01	0.270	0.0080	0.440	0.040	0.024	2.14	0.41	0.26	1.23	0.03	0.02	2.34
	3	9.45	1.070	0.0090	0.230	0.030	0.019	2.38	0.35	0.18	1.33	0.09	0.20	1.74
<i>Cornus florida</i> (dogwood)	1	7.10	0.210	0.0090	0.280	0.040	0.032	2.71	0.47	0.32	1.13	0.14	0.38	2.42
	2	8.01	0.560	0.0070	0.280	0.030	0.034	3.58	0.37	0.06	0.31	0.08	1.54	15.1
	3	12.96	0.610	0.0070	0.240	0.030	0.028	4.21	0.51	0.18	0.37	0.12	0.70	1.37
<i>Ulmus americana</i> (elm)	1	9.40	1.810	0.0060	0.520	0.080	0.010	1.40	0.41	0.50	2.03	0.13	5.93	31.4
	2	10.36	3.270	0.0090	0.810	0.110	0.022	2.42	0.57	0.14	0.20	0.02	2.20	13.8
	3	10.99	3.660	0.0070	0.680	0.130	0.020	2.45	0.53	0.13	0.39	0.11	0.07	2.13
<i>Liquidambar styraciflua</i> (sweet gum)	1	4.83	0.240	0.0150	0.210	0.310	0.002	0.70	0.40	0.37	1.62	0.07	3.02	18.9
	2	5.55	1.030	0.0070	0.740	0.510	0.034	1.55	0.36	0.17	0.71	0.18	0.02	2.63
	3	8.24	1.370	0.0090	0.200	0.700	0.027	1.97	0.43	0.29	0.60	0.07	0.01	1.51
<i>Celtis occidentalis</i> (hackberry)	1	9.15	1.370	0.0080	0.610	0.100	0.029	2.53	0.38	0.44	2.27	0.11	0.22	4.38
	2	10.95	5.460	0.0080	0.730	0.170	0.006	7.60	0.52	0.16	1.73	0.14	0.17	2.93
	3	26.98	5.410	0.0060	0.550	0.170	0.032	7.81	0.53	0.17	1.75	0.10	0.20	2.61

Crataegus polita (haw. red)	1	9.72	0.700	0.0070	0.080	0.0120	0.0034	2.82	0.44	0.26	1.84	0.12	0.182	4.30	15.2	4.4	10.72
	2	8.84	0.510	0.0070	0.060	0.0130	0.0018	3.30	0.50	0.15	1.18	0.10	0.211	5.90	12.1	7.1	14.18
	3	11.98	0.570	0.0010	0.530	0.0150	0.0030	3.70	0.44	0.14	0.92	0.09	0.093	1.70	10.7	7.0	13.28
Ilex opaca (holly)	1	5.25	0.100	0.0010	0.050	0.0260	0.0130	0.54	0.26	0.40	1.08	0.09	0.213	4.90	21.8	6.4	21.4
	2	4.04	0.150	0.0070	0.020	0.040	0.0150	1.07	0.44	0.09	0.91	0.02	0.281	5.10	9.4	4.7	35.5
	3	4.82	0.220	0.0060	0.0270	0.0540	0.0240	0.97	0.31	0.07	0.74	0.09	0.331	0.60	6.8	5.2	33.5
Tilia americana (linden)	1	8.93	0.130	0.0080	0.0220	0.0070	0.0011	1.43	0.40	0.62	2.48	0.42	0.344	72	39.5	3.4	12.3
	2	12.26	0.580	0.0010	0.0430	0.0150	0.0042	5.10	0.77	0.27	1.54	0.20	0.122	45	5.3	10.2	2
	3	19.64	0.540	0.0010	0.0330	0.0210	0.0034	6.43	0.81	0.22	1.12	0.25	11	1.56	11.8	0.3	13.8
Robinia pseudo-acacia (locust)	1	7.87	0.09	0.0000	0.0100	0.0040	0.0007	1.48	0.30	0.56	2.06	0.28	0.395	94	37.2	3.4	10.8
	2	9.19	0.230	0.0070	0.0330	0.0050	0.0047	3.47	0.39	0.29	1.09	0.08	0.053	96	46.8	4	15.3
	3	14.14	0.286	0.0070	0.0330	0.0050	0.0030	4.54	0.41	0.21	1.21	0.00	0.053	112	19.5	4.5	14.0
Acer saccharum (maple)	1	4.80	0.260	0.0010	0.0150	0.0010	0.0024	0.57	0.24	0.46	1.58	0.05	0.243	44	21.5	4.3	10.0
	2	7.20	1.430	0.0010	0.0440	0.0070	0.0016	1.42	0.24	0.00	0.02	0.01	0.11	78	11.8	6.2	16.8
	3	10.30	2.190	0.0010	0.0220	0.0000	0.0034	2.42	0.28	0.30	0.95	0.14	0.011	78	11.1	8.1	16.9
Morus rubra (mulberry)	1	16.73	3.070	0.0070	0.0500	0.0250	0.0030	3.82	0.35	0.20	2.27	0.10	0.093	3.12	19.5	5.9	11.5
Quercus velutina (oak, black)	1	4.20	0.090	0.0000	0.0280	0.0490	0.0038	0.91	0.30	0.15	1.24	0.09	0.222	76	17.3	3.1	25.2
	2	5.10	0.430	0.0000	0.0260	0.1480	0.0068	1.56	0.30	0.17	1.16	0.05	0.252	50	16.2	3.2	23.6
	3	7.65	0.240	0.0070	0.0250	0.1870	0.0066	2.17	0.31	0.20	1.00	0.09	0.042	22	13.9	4.1	22.5
Quercus palustris (normal) (oak, pin)	1	5.20	n.d.	0.0030	0.0180	0.0560	0.0088	1.36	0.25	0.39	1.09	0.70	0.162	33	14.5	n.d.	n.d.
	2	6.58	n.d.	0.0060	0.0450	0.0050	0.0116	1.31	0.21	0.39	1.07	0.75	0.223	36	21.0	n.d.	n.d.
Disopyros virginiana (persimmon)	1	6.13	0.220	0.0080	0.0220	0.0090	0.0042	0.62	0.28	0.46	2.45	0.02	0.284	16	26.0	4.7	12.3
	2	8.40	0.360	0.0000	0.0250	0.0140	0.0048	1.79	0.32	0.17	2.37	0.07	0.282	82	17.6	7.7	12.1
	3	8.79	0.360	0.0030	0.0210	0.0220	0.0036	1.63	0.36	0.14	1.98	0.17	0.272	28	14.3	8.4	11.3
Liriodendron tulipifera (poplar)	1	6.05	0.090	0.0030	0.0240	0.0040	0.0068	1.010	0.33	0.36	1.71	0.04	0.383	88	24.3	5.2	10.4
	2	7.72	0.250	0.0030	0.0380	0.0070	0.0036	3.20	0.30	0.20	1.10	0.01	0.303	01	18.8	4.5	15.0
	3	10.67	0.270	0.0030	0.0280	0.0090	0.0028	3.590	0.21	0.22	0.86	0.07	0.372	81	17.0	5.9	13.3
Platanus occidentalis (sycamore)	1	6.69	0.390	0.0070	0.0210	0.0120	0.0009	1.360	0.30	0.46	1.61	0.41	0.373	49	21.9	2.7	18.3
	2	6.32	0.540	0.0070	0.0180	0.0120	0.0039	1.700	0.31	0.15	1.40	0.04	0.462	44	21.9	2.7	18.3
	3	9.00	0.770	0.0040	0.0260	0.0150	0.0042	2.190	0.30	0.16	1.25	0.10	0.682	07	14.9	3.2	21.9
Juglans nigra (walnut)	1	7.32	0.460	0.0010	0.0280	0.0060	0.0040	1.660	0.35	0.54	2.04	0.09	0.034	57	28.6	4.3	11.0
	2	9.69	0.560	0.0010	0.0780	0.0110	0.0016	2.680	0.30	0.37	2.15	0.16	0.952	18	13.6	4.6	17.4
	3	13.07	0.740	0.0010	0.0490	0.0190	0.0042	3.230	0.50	0.46	1.98	0.15	0.011	74	10.9	5.1	17.4
Cladrastis lutea (yellowwood)	1	8.48	0.230	0.0000	0.0180	0.0030	0.0011	1.850	0.22	0.42	2.44	0.09	0.253	53	22.1	4.5	20.5
	2	9.15	0.780	0.0070	0.0360	0.0050	0.0040	3.230	0.25	0.54	1.81	0.03	0.132	06	16.6	4.1	24.6
	3	13.27	1.900	0.0010	0.0250	0.0070	0.0036	3.680	0.20	0.78	1.88	0.09	0.112	24	14.0	4.4	24.0

* Sample contaminated with copper.

† Sample collected near end of growing season.

‡ Sample collected from trees showing chlorosis due to excess of limestone in soil.

from the copper sulphide was boiled after the addition of 5 ml. of nitric acid to oxidize iron to the ferric condition, cooled, and transferred to a 200-ml. volumetric flask, made to the mark, mixed, and suitable aliquots taken for the determination of iron, manganese, zinc, calcium, magnesium, phosphorus, potassium, and sodium. Separate portions of each sample were weighed out for nitrogen, sulphur, fat, and crude fiber determinations. Moisture was estimated from the loss of weight on drying 10 gm. in air at 100° C. for 5 hours.

Table I contains the analyses. The different samplings are distinguished by the numbers 1, 2, and 3, indicating the order of collection.

Discussion of analyses

The average total water content of the first lot of samples was approximately 75 per cent; that of the second, approximately 60 per cent; and that of the third, approximately 55 per cent. These results demonstrate that as the growing season advanced the water content of the leaves decreased appreciably.

Excepting the holly, the percentages of ash of the three lots of leaves showed a very marked increase as the season advanced. In the third sampling the maximum percentage of ash was 26.98 (in the leaves of the hackberry) and the minimum, 7.65 (in the leaves of the black oak).

The different species of trees showed considerable variation in the percentage of silica in their leaves at different times during the growing season. The maximum percentage of silica was 5.41, in the hackberry leaves. Other species, in descending order of percentage of silica in the dry leaves, were elm, mulberry, maple, yellowwood, buckeye, and sweet gum. In the remaining 15 species the silica was less than 1.2 per cent.

Copper is a constant and rather uniform constituent in the leaves, but was found in smaller quantities than any of the other elements determined. The catalpa leaves contained the largest quantity of copper, and it remained practically constant in the leaves as the season advanced. The smallest amount of copper occurred in the leaves of the yellow poplar, which contained about one-fifth as much as the catalpa leaves. Copper does not vary much in the

different leaves analyzed, and the maximum amount is considerably less than either manganese or zinc, the latter elements showing considerable differences, both seasonal and among the different species. Rocks and soils contain relatively small amounts of copper, some of which is absorbed through the roots and deposited in the tissue of the plants. From its constant and uniform occurrence in the leaves, it is to be assumed that copper has an important function in the metabolism of trees.

The iron content of the different species of leaves shows considerable variation. It is greatest in elm, black walnut, hackberry, catalpa, and mulberry. The samples collected at mid-season contained appreciably more iron than those collected at the beginning and at the end of the growing season. Of the four metallic elements copper, manganese, zinc, and iron, the last occurs in the greatest amount in all species except sweet gum, black oak, and pin oak. In the leaves of these three species the manganese content considerably exceeds the iron content. From previous experiments with lower types of plants, it seems likely that each of these elements has important functions in the economy of the higher plants.

The leaves of different species of trees show considerable variation in the amount of manganese they contain. The pin oak, sweet gum, black oak, and elm contain relatively large amounts of manganese. The leaves of these trees have a characteristic deep green, glossy appearance during the growing season, which indicates that they contain a larger amount of chlorophyll than the leaves of other trees which have a lighter green color. This fact indicates a correlation between manganese content and chlorophyll. There is considerably more manganese than iron in the leaves of the sweet gum, pin oak, and black oak, whereas the iron content of the leaves of other species analyzed exceeds the manganese content. The leaves of these three species develop beautiful autumnal colors, and it is assumed that an excess of manganese over iron is probably a factor in the production of these colors. The manganese content of the leaves of nearly all the species increases as the growing season advances. QUARTAROLI (3) reports results which show that the manganese content of leaves increases with their age, and our results are in accord with his findings.

It has also been observed that when young pin oak and sweet gum trees are set in a soil containing considerable calcium carbonate, the leaves become chlorotic for several seasons and the trees die. Apparently these trees require an acid soil for a normal growth.

Zinc is a normal constituent of all of the different species of leaves analyzed; however, the amount contained in the different samplings shows some wide variations. The maximum percentage of zinc occurred in the holly leaves and the minimum in the leaves of the elm. Some of the leaves showed a gradual accumulation of zinc during the growing period. In a few species the young leaves contained a greater percentage of zinc than the mature leaves.

The calcium content of all the leaves except the crabapple, holly, and persimmon increased as the season advanced. The greater increase of this element in the leaves occurred during the interval between the first and second samplings. The maximum calcium content, 7.8 per cent, was found in the mature leaves of the hackberry. This is equivalent to 390 pounds of calcium carbonate per ton of the dry leaves. The minimum calcium content of any of the leaves, except holly, at the end of the growing season was found in the leaves of the persimmon. It was 1.63 per cent, which is equivalent to 81.5 pounds of calcium carbonate per ton of dry matter, a little less than one-fourth of the calcium carbonate equivalent of the hackberry leaves. YANOVSKY, NELSON, and KINGSBURY (7) have recently shown that the pits of hackberry seeds contain considerable calcium carbonate; it is unusual for calcium carbonate to occur in plants. Qualitative tests on hackberry pits from trees on the grounds of the Kentucky Agricultural Experiment Station on June 1, 1932, confirm their findings. The hackberry tree apparently assimilates a greater amount of calcium than any other of the species analyzed.

Under natural conditions it has been observed that certain species of trees are adapted to certain types of soil. In eastern Kentucky, where the soils have been derived from the disintegration of sandstones and shales and are low in calcium, the most common species of forest trees are black, white, and chestnut oaks, yellow poplar, ash, sweet gum, dogwood, wild cherry, red and yellow pine, beech, chestnut, sassafras, and persimmon; whereas in central Kentucky, where the soils have been derived from limestone formations, the

more common species include black walnut, burr oak, sycamore, sugar maple, hickory, locust, elm, hackberry, coffee bean, and ash.

The average calcium content of the leaves of seven species of trees common in central Kentucky, black walnut, sycamore, sugar maple, locust, catalpa, linden, and hackberry, is 4.1 per cent; whereas the average of seven species, black oak, sweet gum, yellow poplar, dogwood, wild cherry, persimmon, and ash, whose natural habitat is a sandstone soil, is 2.5 per cent. The latter trees were growing in a soil derived from limestone, however, and probably contained more calcium than leaves of the same species growing in a soil low in calcium, which is their normal habitat.

The magnesium content of the leaves of all species is remarkably constant throughout the growing season. In the majority of the samples there is a slight increase in the magnesium content as the season progresses. The two species which contained the greatest amount of calcium also contained more magnesium in proportion than the leaves which were low in calcium.

The phosphorus content in one-third of the samples of the young leaves ranged from 0.62 to 0.54 per cent, while in the remaining samples collected at the same time the range for phosphorus was 0.48 to 0.15 per cent. The leaves which contained the greatest amounts of phosphorus are, in the order named, linden, buckeye, ash, elm, catalpa, locust, and black walnut. The yellowwood, black oak, and crabapple leaves are abnormal with respect to phosphorus in that the last samples of the season contain more phosphorus than the first. In all the other species the young leaves contained the greatest percentage of phosphorus. The maximum amount of phosphorus in any of the young leaves was 0.62 per cent, which is equivalent to 12.4 pounds of phosphorus per ton of dry matter. The maximum phosphorus in the last sampling was 0.78 per cent, or 15.6 pounds of phosphorus per ton of dry matter. The minimum phosphorus, 0.2 per cent, occurred in the leaves of the black oak.

The sodium content of all the leaves was low and rather constant in amount. Although sodium is universally present in plant material, it has not been shown to have any necessary function in the economy of plants.

The percentage of potassium in the leaves of all the species was

greatest in the young leaves and diminished to about 50 per cent of the maximum by the end of the growing season. In a few species the maximum potassium content was twice as much as in others, but in the majority of the leaves the differences were less.

In most of the species the sulphur content was greatest in the young leaves and gradually diminished in the second and third samples. In the dogwood and catalpa, however, the third sample (the oldest leaves) contained nearly twice as much sulphur as the first sample. The persimmon leaves contained approximately the same amount of sulphur in each of the three samples. In the leaves of the linden the first sample contained a normal amount of sulphur but the last contained only a trace.

The nitrogen content of the leaves of the different species of trees affords several points of interest. The young leaves of the locust contained the greatest percentage of nitrogen of any of the species. The young leaves of the ash and the elm contained nearly as much nitrogen as the locust, although they do not belong to the family of legumes. The mature leaves of the locust contained 3.12 per cent of nitrogen, which is equivalent to 62.4 pounds of nitrogen per ton of moisture-free material. This amount is comparable with that contained in the leguminous forage crops.

The ether extract includes resins, chlorophyll, oily and fatty substances. Probably resins constitute a greater part of the ether extract than either chlorophyll or fatty material. The crabapple leaves contained considerably more ether extract than any of the other leaves. The ether extract from the crabapple leaves was a rather firm, compact solid, however, which led to the assumption that it contained considerably more resinous matter than fatty material. In most of the leaves the last samples contained a greater amount of ether extract than the first samples; however, the extract from the second sample of dogwood leaves was nearly twice as much as the extracts in either the first or third samples. In a few of the species the ether extract was less in the second samples than in either the first or third samples.

The crude fiber determinations show that the leaves can be put into three groups, low, medium, and high. The leaves that have a low crude fiber content, 15 per cent or less, include crabapple, ca-

talpa, cherry, dogwood, elm, gum, hackberry, haw, linden, locust, persimmon, and poplar. When dry, the leaves of these trees are quite brittle and they disintegrate rapidly in the soil. The leaves which have a medium crude fiber content, approximately 20 per cent, include the ash, cucumber, maple, sycamore, and walnut; and the species which have a high crude fiber content are buckeye, holly, black oak, and yellowwood. The leaves in the second and third groups would disintegrate more slowly in the soil than those in group one.

TABLE II

AVERAGE COMPOSITION OF LEAVES OF 21 SPECIES OF TREES COLLECTED AT THREE INTERVALS DURING GROWING SEASON OF 1931; RESULTS CALCULATED AS PERCENTAGES OF MOISTURE-FREE MATERIAL

	FIRST SAMPLING MAY 4-8	SECOND SAMPLING AUGUST 3-6	THIRD SAMPLING SEPTEMBER 4-6	NUTRIENTS (LB. PER ACRE)*
Ash (crude).....	7.150	8.459	11.449	458.00
Insoluble residue (SiO ₂).....	0.376	0.951	1.159	46.40
Copper (Cu).....	0.001	0.0009	0.0009	0.04
Iron (Fe).....	0.029	0.0429	0.0337	1.35
Manganese (Mn).....	0.011	0.0209	0.0205	1.06
Zinc (Zn).....	0.003	0.0042	0.0046	0.18
Calcium (Ca).....	1.329	2.767	3.142	125.68
Magnesium (Mg).....	0.353	0.422	0.404	16.16
Phosphorus (P).....	0.452	0.234	0.274	10.96
Potassium (K).....	1.997	1.344	1.173	46.90
Sodium (Na).....	0.165	0.087	0.116	4.60
Sulphur (S).....	0.281	0.187	0.170	6.80
Nitrogen (N).....	3.829	2.365	1.970	78.80
Protein (N×6.25).....	23.930	14.78	12.310	492.40
Ether extract.....	4.910	5.57	6.210	248.30
Crude fiber.....	13.690	18.12	16.990	679.80

* DAY (1) assumes an annual leaf-fall in forests of more than 2 tons per acre.

There seems to be a very general impression among plant physiologists and botanists that toward the end of the growing season there is a gradual transfer of plant nutrients from the leaves back to the branches, trunk, and roots of trees. The averages of the different constituents in the first, second, and third samplings were computed to ascertain whether there were any trends among the different constituents determined confirming this very general idea. Table II contains the averages of the different constituents for the three different samplings. The results are percentages of the moisture-free material.

In table II it is to be observed that the ash, insoluble residue, manganese, zinc, calcium, and ether extract showed a decided increase in the leaves as the season advanced, whereas potassium, sulphur, and nitrogen showed a gradual decrease during the growing season. Copper remained practically unchanged; iron, magnesium, and crude fiber attained a maximum in the second sampling and declined slightly in the third. Phosphorus declined nearly 50 per cent between the first and second samplings and regained appreciably in the third sampling. Nitrogen, potassium, and sulphur are the only elements that showed a slight diminution in the interval between the second and third samplings. It is assumed that this diminution is only apparent and is due principally to the formation of cellulose and other compounds which dilute the potassium and nitrogen content as the season advances. These results do not afford any convincing evidence that there is a migration of plant nutrients from the leaves to the branches of trees toward the latter part of the growing season.

It is reported that in some European countries the leaves of certain species of trees are gathered and used as a substitute for hay. In this country, however, no such practice is common. In the spring, when the leaves of trees are young and tender and grass scarce, cattle will feed on forest leaves, but other livestock apparently do not relish this kind of forage under ordinary circumstances.

Summary

1. The leaves of 23 species of deciduous trees were analyzed at three intervals during the growing season. The results show that the dry matter of young leaves contained the largest percentages of phosphorus, potassium, and nitrogen, whereas the mature leaves collected at the end of the growing season contained the largest percentages of ash, silica, and calcium. Copper, manganese, and zinc were normal constituents in all of the samples. In three of the species, sweet gum, black oak, and pin oak, the manganese content was in excess of the iron content, which is an unusual relationship. The leaves of these trees develop beautiful colors during the late autumn season, and the unusual relationship of iron and manganese may be a factor in the production of these autumnal shades.

2. The results do not afford material evidence that mineral nutrients migrate from the leaves to the branches toward the end of the growing season.

3. A mixed forest probably would add as much plant nutrient to the surface soil in an annual crop of leaves as is removed by the average forage crop.

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CYTOLOGY OF ANCHUSA AND ITS RELATION TO THE TAXONOMY OF THE GENUS

STANLEY G. SMITH

(WITH SEVEN FIGURES)

Introduction

The great diversity of form to be found within the genus *Anchusa*, together with the clear-cut differences exhibited between the species, suggested that the cytology of the genus might be interesting. After a preliminary study of several of the common species, it was so surprising to find in *A. myosotidiflora* Lehm. a chromosome complex differing entirely from any previously seen, that Dr. O. STAFF, of the Royal Botanic Gardens, Kew, England, was consulted as to the validity of its classification. It was gratifying, therefore, to learn that recently, agreeing with JOHNSTON (9), STAFF (15) had published a description of the plant, treating it as a member of the restored genus *Brunnera*.

According to JOHNSTON, this species was originally described by MARSCHALL (13) as *Myosotis macrophylla*; then by LEHMANN (11) as *Anchusa myosotidiflora*. STEVEN (16) separated it from the genus *Anchusa* and assigned it to the closely related and newly described genus *Brunnera*, calling it *B. myosotidiflora*. This splitting of the genus, however, was not accepted until the publication of JOHNSTON's study of the Boraginaceae (9). He came to the conclusion that *Anchusa myosotidiflora*, with its variety *grandiflora*, and *A. neglecta*, plants indigenous to Siberia and the eastern Mediterranean region, exhibited sufficient differences to warrant their treatment as generically distinct from *Anchusa*. Following the law of priority for the specific epithet, the name of *Anchusa myosotidiflora* automatically became *Brunnera macrophylla* (Marschall) Johnston.

Among the material examined before JOHNSTON's paper was received, *Anchusa sempervirens* L., a species barely justifying its inclusion in the genus on account of its distinct morphology, was found to possess a complement of chromosomes so markedly dis-

similar in size and number from all others up to this time examined that, on the analogy of *Brunnera macrophylla*, it also seemed to merit its early recognition as the distinct genus *Caryolopha* Fisch. and Traut (5).

A tabular comparison of JOHNSTON'S arrangement with that of GÜRKE (6) follows. In the cytological observations, the nomenclature used throughout is that given by the latter investigator. The species are discussed in the order in which they appear here.

ANCHUSEAE

	GÜRKE'S CLASSIFICATION	JOHNSTON'S CLASSIFICATION
Anchusa	Section I, Buglossum	{ A. barrelieri A. ochroleuca A. italica }
	Section II, Buglossoides	
	Section III, Euanchusa	{ A. hybrida A. officinalis }
	Section IV, Caryolopha	
	Section V, Myosotoides	A. sempervirens Caryolopha A. myosotidiflora Brunnera macrophylla

Material and methods

A list of the plants examined is as follows.¹ They were kindly supplied by the directors of the various institutions mentioned.

Anchusa sempervirens	Royal Horticultural Society, Cambridge Botanical Gardens, John Innes Horticultural Institution
A. myosotidiflora	Royal Horticultural Society
A. barrelieri	Chelsea Physic Gardens
A. ochroleuca (seeds)	Valencia
A. capensis	John Innes Horticultural Institution
A. hybrida	John Innes Horticultural Institution
A. italica	John Innes Horticultural Institution
A. italica var. Pride of Dover	John Innes Horticultural Institution
A. italica var. Dropmore	John Innes Horticultural Institution
A. italica var. Opal	John Innes Horticultural Institution
A. officinalis	Royal Botanic Gardens

¹ Illustrations of these plants may be seen in the following volumes of CURTIS'S Botanical Magazine: *A. barrelieri* 49 tab. 2349, *A. capensis* 43 tab. 1822, *A. italica* 48 tab. 2197, *A. ochroleuca* 39 tab. 1608, *B. macrophylla* 151 tab. 9110. *C. sempervirens* is shown by GUSULEAC in Repert. Spec. Nov. Fedde 29:42-47. 1931.

Plants were grown in pots and root tips were fixed in LA COUR'S (10) 2BE fixative. Material was cut at 10, 14, and 20 μ , the thickness varying with the size and number of chromosomes, and stained by a chromic acid modification of Newton's gentian violet method (LA COUR 10).

Somatic chromosomes

In Section I, Buglossum, six plants (*A. barrelieri*, *A. ochroleuca*, *A. italica* and three of its garden varieties) were examined for somatic chromosome number and form.

Anchusa barrelieri (fig. 1) was found to be diploid with $2n=16$ chromosomes, of which one type with a subterminal attachment constriction, another with a slight almost terminal secondary constriction, and the smallest of the complement with a median point of attachment, were present in duplicate only. The remaining types were difficult to determine with any degree of certainty, but, taking other cells into consideration, the whole complement seemed to be:

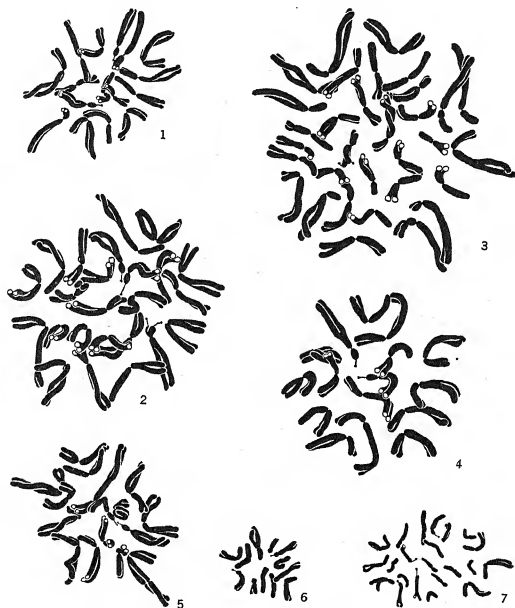
- 1 pair with a subterminal constriction and a trabant on the shorter segment;
- 2 pairs with median constrictions;
- 5 pairs with submedian constrictions.

Anchusa ochroleuca seedling (fig. 2) arose from seeds received from Valencia and gave a somatic count of 24 chromosomes. This plant has a complement of chromosomes in triplicate, the most obvious of which are the three subterminally constricted chromosomes bearing trabants. The others without doubt are present also in triplicate, although this is not apparent in the figures, owing to their large size and contorted shape and the limitations of two-dimensional representation. The arrangement of the chromosomes with regard to their points of attachment was found to be:

- 3 chromosomes with subterminal constrictions and trabants on the minor segments;
- 6 chromosomes with median constrictions;
- 15 chromosomes with submedian constrictions, one of which had a slightly secondary constriction.

This triplicate constitution indicates that the seedling is an auto-triploid derived from the fertilization of an abnormal diploid ovule

by a normal haploid gamete, or vice versa. The former seems to be the more probable in the light of research on tetraploid *Datura* (BLAKESLEE, BELLING, and FARNHAM 3).



FIGS. 1-7.*—Somatic metaphase plates from root-tip cells of seven species of the Anchuseae: fig. 1, *Anchusa barrelieri* ($2n=16$); fig. 2, *A. ochroleuca* ($2n=24$); fig. 3, *A. italica* var. *Pride of Dover* ($2n=32$); fig. 4, *A. officinalis* ($2n=16$); fig. 5, *A. hybrida* ($2n=16$); fig. 6, *Brunnera macrophylla* (syn. *A. myosotidiflora*) ($2n=12$); fig. 7, *Caryophylla sempervirens* (syn. *A. sempervirens*) ($2n=22$). $\times 4200$.

*All drawings made with the aid of a camera lucida, a Zeiss 2 mm. objective, N.A. 1.3, $30\times$ ocular.

Unfortunately this plant and its sibs were inadvertently destroyed before the cytological material was examined, and there is therefore no way of determining whether or not the triploid condition was exceptional. Nevertheless, it seems particularly significant that the one plant chosen as a typical healthy representative of a batch of seedlings should, throughout all the root tips, exhibit triploidy with which is usually associated a certain degree of dissimilarity from the true diploid. It is hardly possible that this plant was a segregate from a triploid, as the chance of two gametes combining to restore a triply balanced set of chromosomes is extremely rare (BELLING 2), even in the first generation from a newly arisen triploid.

Anchusa italica and its varieties Pride of Dover, Dropmore, and Opal have $2n=32$ chromosomes. In Pride of Dover (fig. 3) the following types of chromosomes were noted:

- 2 pairs with subterminal constrictions, the smaller pair bearing trabants on the major arms;
- 3 pairs with median constrictions;
- 11 pairs with submedian constrictions, including one with a secondary constriction situated on the minor segment close to the point of attachment. The longest pair of chromosomes, which were in this class, had a slight secondary constriction about half-way along the major arm.

In all these plants only two chromosomes of each distinguishable shape were present, as in *Sorghum halepense* (HUSKINS and SMITH 8), which very strongly suggests that they are of an allotetraploid origin similar to that of *Primula kewensis* (NEWTON and PELLEW 14).

In the variety Opal there was a fragmented chromosome. This was probably additional to the normal tetraploid complement, but difficulty was experienced in obtaining a preparation sufficiently well fixed to show the entire set.

Of the species included in Section III, *Euanchusa*, three (*A. officinalis*, *A. hybrida*, and *A. capensis*) were examined. Two are illustrated here, and the third, *A. capensis*, had an apparently similar complement.

Anchusa officinalis (fig. 4) was a normal diploid with 16 somatic chromosomes, which could be classified as:

- 1 pair with a subterminal constriction and a trabant on the minor arm;
- 3 pairs with median constrictions, one of which had a subordinate, almost terminal, constriction which was less obvious and only occasionally observed;
- 4 pairs with submedian constrictions, including one pair of much greater length than the others.

Anchusa hybrida (fig. 5) possessed a complement of 16 chromosomes strikingly similar to *A. officinalis* and *A. capensis*. The eight different types could be arranged as:

- 1 pair with a subterminal constriction and a trabant² which was again situated on the minor arm;
- 3 pairs with median constrictions;
- 4 pairs with submedian constrictions. The longest pair possessed a sharp secondary constriction about half-way along the major arm, which divided the chromosome into three almost equal segments. (An indication of the same condition is to be observed in a comparable chromosome of the *A. ochroleuca* seedling illustrated in figure 2.)

Section IV, Caryolopha.—This is a monospecific section, having *A. sempervirens* (*Caryolopha sempervirens*) as its sole representative. Somatic counts gave a diploid complement of 22 chromosomes (fig. 6), all of which were decidedly smaller than those of the previous sections. Some of the morphological types seemed to be present in quadruplicate, although, as is obvious from the illustration, it would be inadvisable to state this definitely. These chromosomes also could be arranged with regard to their points of attachment into three classes:

² Other cells were seen in the same root tip in which each chromosome of this pair bore a trabant on the shorter segment, but the trabants were so small that difficulty was encountered in finding them in every cell, even though their position was known. These elements have therefore been included in the drawing, although they were not observed in this particular cell.

- 2 pairs with trabants and subterminal constrictions, one pair of which had a secondary constriction at the end distal to the trabant;
- 3 small pairs with median constrictions;
- 6 pairs with submedian constrictions, including one very large pair.

Anchusa myosotidiflora (*Brunnera macrophylla*), the only species examined in GÜRKE's Section V, *Myosotoides*, was found to have $2n=12$ chromosomes (fig. 7), all of which had approximately the same mass as those of the preceding section. The members of this complement were found to fall into the following classes:

- 2 pairs having subterminal constrictions, the larger of which was in accordance with the general rule in that it possessed a trabant on the minor arm;
- 1 pair with a median constriction;
- 3 pairs with submedian constrictions.

Discussion

Table I compares the two systems of classification diagrammatically in relation to the cytological results. It is particularly

TABLE I

GÜRKE'S CLASSIFICATION	2n CHRO- MO- SOME NUM- BER	CHROMOSOME TYPES CLASSIFIED ACCORDING TO POSITION OF POINT OF ATTACHMENT			CHROMOSOME LENGTH (μ)		JOHNSTON'S CLASSIFICATION
		SUB- TERMI- NAL	MEDI- AN	SUB- MEDI- AN	SHORT- EST	LONG- EST	
<i>Anchusa barrelieri</i>	16	2	4	10	4.2	6.1	<i>Anchusa barrelieri</i>
<i>A. ochroleuca</i> seedling	24	3	6	15	5.5	8.0	<i>A. ochroleuca</i> seedling
<i>A. italica</i> var.							<i>A. italica</i> var.
Pride of Dover	32	4	6	22	4.7	8.5	Pride of Dover
<i>A. officinalis</i>	16	2	6	8	5.9	8.5	<i>A. officinalis</i>
<i>A. hybrida</i>	16	2	6	8	5.2	7.8	<i>A. hybrida</i>
<i>A. sempervirens</i>	22	4	6	12	1.8	3.8	<i>Caryolophia semper- virens</i>
<i>A. myosotidiflora</i>	12	4	2	6	1.4	3.0	<i>Brunnera macro- phylla</i>

noticeable that the more important changes in classification occur with plants which are macroscopically different from the type most

generally accepted by horticulturists and systematists to be *Anchusa*, and which appear to be of doubtful classification from a consideration of their microscopic differences, both in external and internal morphology.

From a consideration of its chromosomal constitution, we may safely regard *Anchusa italica* as a new and relatively constant form evolved by hybridization and subsequent doubling of the hybrid complex like *Spartina townsendii* (HUSKINS 7) and other cases. One might therefore consider this as concrete support for JOHNSTON's assumption that phylogenetic relationships in this group of plants are reticular rather than dendritic. The cytology also justifies JOHNSTON's action in combining the two sections, Buglossum and Euanchusa.

On cytological grounds the segregation by JOHNSTON of both *Anchusa myosotidiflora* and *A. sempervirens* into other genera as *Brunnera macrophylla* and *Caryolopha sempervirens* is completely vindicated. They show a dissimilarity in size and basic number existing between them and the genus to which they had previously been referred. But although these obvious differences in nuclear constitution occur, there are nevertheless one or two strikingly similar chromosome forms observable in all three genera. For example, the trabanted subterminally constricted chromosome persists, with one exception (*Anchusa italica* var. *Pride of Dover*, fig. 3), throughout the plants examined, and, further, the longest pair of chromosomes is represented in nearly all the species with an occasional modification in the form of a secondary constriction (figs. 3, 5). In view of the increasing evidence indicating that chromosome size or structure may be governed by genes (LESLEY and FROST 12, DARLINGTON 4, and HUSKINS and ARMSTRONG, unpublished), it seems reasonable to assume that gene mutation may have been responsible for some of the differences in the present case.

Although we are not here concerned with specific ranking, the question might be raised whether, on the analogy of *Brunnera* and *Caryolopha*, the extreme similarity among the chromosome complexes of *A. barrelieri*, *A. capensis*, *A. hybrida*, and *A. officinalis* indicates a necessity for grouping them all into one species. But as BABCOCK (1) points out, specific differences are not necessarily ac-

accompanied by visible changes in the chromosomes; or, in other words, while generic status is usually accompanied by definite chromosome variation, specific differentiation is more often effected by an accumulation of genic differences having no visible effect on gross chromosome structure.

Summary

1. *Anchusa italica* and its varieties are shown to be allotetraploids. This is in accord with JOHNSTON's theories of reticular phylogenetic relationships in the Boraginaceae.

2. The following chromosome numbers were found: *Anchusa barrelieri* $2n=16$, *A. capensis* $2n=16$, *A. hybrida* $2n=16$, *A. officinalis* $2n=16$, *A. ochroleuca* $2n=24$, *A. italica* $2n=32$, *A. italica* vars. Pride of Dover, Dropmore, and Opal $2n=32$, *Brunnera macrophylla* $2n=12$, and *Caryolopha sempervirens* $2n=22$.

3. The origin of the triploid seedling of *Anchusa ochroleuca* is discussed.

4. The removal by JOHNSTON of *Brunnera macrophylla* from the genus *Anchusa* is substantiated by the number and character of its somatic chromosomes.

5. The similar systematic treatment of *Caryolopha sempervirens* is also supported by cytological observation.

6. JOHNSTON's combining the sections Buglossum and Euanchusa is in agreement with their cytological similarity.

This study was carried out chiefly at the John Innes Horticultural Institution, Merton, England, being initiated at the suggestion of the Director, Sir A. D. HALL. The author wishes to express his indebtedness to Dr. C. LEONARD HUSKINS and Dr. EDGAR ANDERSON for their valuable criticisms of the manuscript.

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FASCIATION IN RED PINE

RAYMOND KIENHOLZ

(WITH TWELVE FIGURES)

Introduction

The peculiar flattening, or fasciation, of normally cylindrical shoots is frequently encountered in herbaceous plants. Notable in this respect is *Celosia cristata*, whose fastigate character is inheritable. Woody plants seem to exhibit fasciation less frequently than herbaceous plants, and conifers less frequently than broad-leaved trees. Among conifers, spruce seems particularly subject to fasciation and pines less so. Early in the study of the phenomenon of fasciation it was believed that the thin, flat twigs were caused by the growing together of several twigs, but this idea is no longer held. Some internal physiological disturbance causes the growing *point* to become a growing *line*, resulting in the production of flattened twigs. The cause and nature of this physiological disturbance are not known. Pressure experimentally applied to the growing tip has not produced fasciation. TANNER (7) believes fasciation to be associated with over-nutrition.

A considerable amount of literature is devoted to the description of fasciation, but the great majority of this work, particularly as it applies to conifers, is European. An easily procurable, general account of fasciation may be found in MASTER'S (4) well known work on vegetable teratology. SCHENCK (5) compiled much information dealing with fasciation in conifers and published several fine illustrations showing the flattening, bending, torsion, and abundant production of twigs characteristic of this phenomenon. TANNER has more recently described and figured fasciation in forest trees. In this country fasciation in conifers has apparently seldom been observed. HUBERT (3) figured a fasciated twig of larch, and BALDWIN (1) described a fasciated leader of Scotch pine which showed the characteristic flattening (22 mm. wide by 10 mm. thick), the division into two, the bending due to differential growth, and the large number of

buds at the tip. Fasciation has also been observed in Douglas fir, western hemlock, and Sitka spruce.

Even in Europe, however, fasciation occurring over several successive years seems to be very rare, since SCHENCK (6) gave as his reason for adding to the "already over-rich literature" the fact that so far only a few cases of fasciation extending over several successive years had been described in an exact morphological manner. He described (5) fasciation extending over successive years in *Araucaria cunninghamii*. He also cites DE VRIES (2), who described fasciation affecting six successive years of growth of a specimen of *Picea*. TANNER cites examples of fasciation extending over several years.

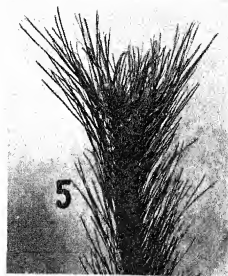
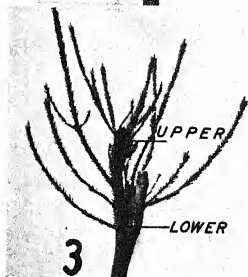
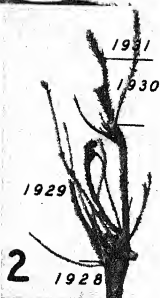
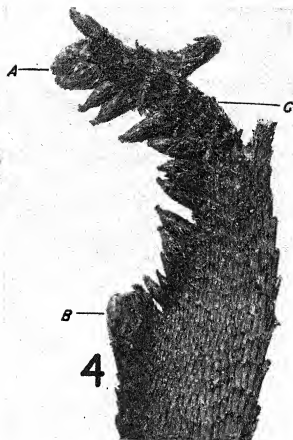
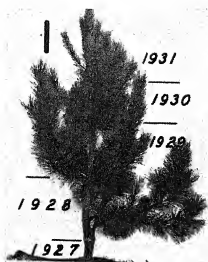
This phase of the subject is of particular interest, since little information is available concerning the length of life of fasciated branches after the phenomenon first appears. Likewise, whether fasciation continues to manifest itself each year after its first appearance is unknown.

Observations

The fasciation here described occurred in the leader and upper laterals of a specimen of *Pinus resinosa* during five years (1927 to 1931) of its growth. This pine was growing in an open plantation spaced 8 by 8 feet on a gently sloping southwest exposure, located in the Yale Demonstration and Research Forest near Keene in southern New Hampshire. The soil is a fine sandy loam with a fine gravelly subsoil, moderately well watered. The crowns of the trees in the plantation were just beginning to touch and in a few cases the lower limbs had begun to die. The fasciated pine was 18 years old and about 6 meters high.

In October 1931, the upper part of the tree was cut off, photographed, and measured. The upper fasciated portion of the tree (fig. 1) was of a very dense, bushy appearance. The first distinct appearance of fasciation occurred in the 1928 leader, but even below this the number of lateral branches per whorl was greater than that of the surrounding normal trees. Table I gives the number of laterals per whorl for the fasciated tree in comparison with the average of five normal trees growing nearby.

A section through the upper part of the 1927 leader (fig. 8) showed the first annual ring to be distinctly flattened (13 by 5.5 mm.),



FIGS. 1-5.—Fig. 1, general view of fasciated growth, with the 1928 laterals removed, the whole about 1.7 meters tall. Note bushy, leafy appearance of fasciated growth and the overtopped 1928 leader to right. (Same as fig. 6.) Fig. 2, overtopped 1928 leader, with needles removed, showing flattening and excessive production of twigs. (Same as

being over twice as broad as it was thick. Later, annual growths tended to round out the stem, and the diameters of this section in 1931 were 67 and 63 mm.

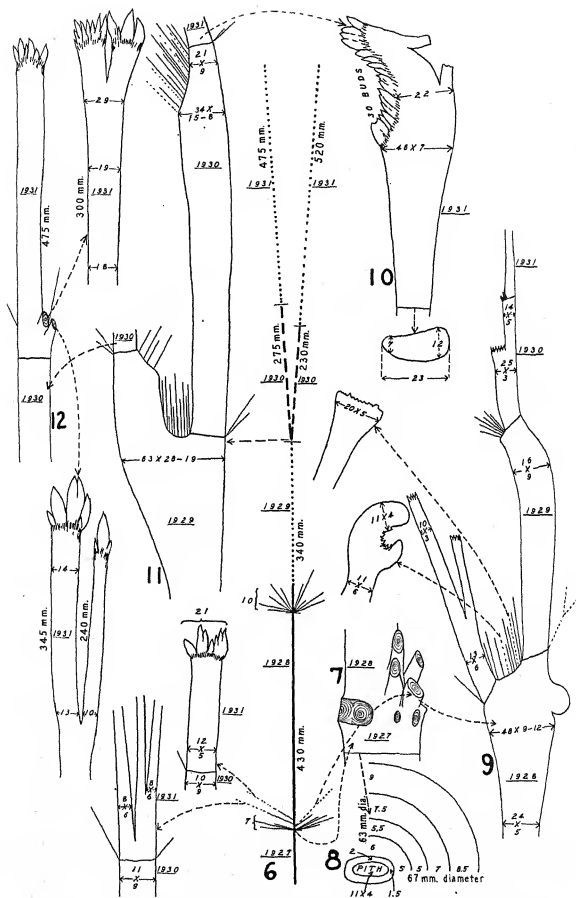
TABLE I

	1927	1926	1925	1924	1923	1922	1921	1920
Fasciated.....	8.0	8.0	4.0	6.0	5.0	5.0	4.0	3.0
Normal.....	4.8	3.6	3.6	3.2	3.4	3.0	2.8	2.4

The 1928 leader as it developed became distinctly fasciated, producing a greatly flattened tip (48 mm. wide by 9 mm. thick on one edge and 12 mm. thick on the other edge) bearing many buds (figs. 2, 9). The next year many of these buds grew out into twigs, some of which showed fasciation, others not. The buds at either edge of the broad flat tip were the stronger and produced larger, more vigorous, usually fasciated twigs that grew for several years. The buds in between produced smaller twigs which were more apt to be normal. The fasciated twig to the right produced six small twigs in 1930 and none in 1931; and the buds for 1932 were so small and dry as to appear dead. The whole leader was so completely overtopped and so weakened in its growth that it was probably only a matter of a few years before it would have died.

A normal-appearing 1928 lateral bent up to take the place of the fasciated 1928 leader. This new leader, however, exhibited extensive fasciation in 1929 and in later years (figs. 3, 11). Its greater vigor was shown by its length growth during the years 1929, 1930, and 1931. Here again the broad, flattened tip of the 1929 growth (63 mm. wide by 28-19 mm. thick) produced two larger vigorous shoots both of which developed strongly, causing a forking of the

fig. 9.) Fig. 3, part of the new 1928 leader, with needles removed, showing lower mass of twigs produced in 1930 and upper mass produced in 1931. (Same as fig. 11.) Fig. 4, detailed view of example of extreme fasciation in 1931 twig (needles removed). Edges of twig are thicker than middle, which is slightly dished. Largest buds are borne on the edges (A and B). More rapid growth on right-hand edge is shown by greater distance between the leaf scars and by position of the bud-bearing surface. Stresses set up by unequal growth resulted in splitting away of the tissue at C. (Same as fig. 10.) Fig. 5, moderately fasciated twig (1931 growth) showing forking at tip, and excessive bud and needle production. (Same as twig shown at right and above in fig. 12.)



FIGS. 6-12.—Diagrammatic and semidiagrammatic representation of fasciated growth, not drawn to scale; all dimensions in mm. Fig. 6, diagram of entire fasciated growth showing years and lengths. Fasciated growth indicated by dotted and dashed

tree. Between these two shoots ten small twigs were produced. These are shown in fig. 3 as the lower mass of twigs. The branch to the right continued flattened in 1930 (34 mm. wide by 15-18 mm. thick) and produced 16 small twigs in 1931, some fasciated, others not (upper twig mass in figs. 3 and 11), and one large vigorous twig which produced the most extreme example of flattening found on the tree (figs. 4, 10). The branch to the left produced a vigorous fasciation (fig. 12).

The extreme flattening of the 1931 twig on the right is shown by its dimensions of 46 mm. broad and an average of 7 mm. thick (fig. 4). It is slightly thicker at either edge and somewhat dished in the middle. The needles borne on this central dished portion are shorter than those borne along the two edges. They are nearly all normal in number, that is, two per fascicle. The total number of needles produced on a fasciated twig seems to be greater than on a normal twig, which, together with their greater crowding, caused the fasciated twig to assume a very bushy appearance. The fact that the two edges of the twig are thicker than the middle may account for the production of more vigorous buds at those points.

Many of the fasciated twigs are characterized by a more rapid growth along one edge than along the other, causing the bud-bearing tip to assume a shape such as that shown in figure 4. Rapid and unequal growth accompanied by bending caused the cracking and splitting away shown at C in figure 4. This condition is frequently seen in cases of extreme fasciation in herbaceous plants.

lines, normal growth by solid lines. Note laterals at base of 1928 and 1929 growth of leader. Arrows lead to portions shown in detail. Fig. 7, base of new 1928 leader showing origin of several laterals at different levels and the overtopped 1928 leader. Fig. 8, section through upper part of 1927 leader showing greatly flattened pith and first-year wood. Later growths tended to round up the section somewhat. Diameters at end of 1931 were 63 and 67 mm. Fig. 9, overtopped 1928 leader showing unequal forking due to production of most vigorous twigs at the two ends of growing line. Twigs produced between (10 in number) are not drawn to scale, but fasciation is shown by dotted lines, normal twigs by solid lines. Fig. 10, extreme fasciation (1931 twig) showing dimensions, buds, and splitting at upper edge. Section through base of this growth shows width of 23 mm., and thickness at one edge of 7 mm. and at other edge of 12 mm. Fig. 11, new 1928 leader showing forking at beginning of 1930 growth with the two edges producing strong twigs. Numerous (10) smaller twigs produced between and also at base of 1931 growth (16 twigs). Fig. 12, twigs (1931) showing moderate fasciation.

Discussion

Apparently the effects of this physiological disturbance within the tree became more severe from the time of its first pronounced appearance (1928) to the time when the specimens were collected (October, 1931). Although the 1927 leader appeared normal at the time of collection, a section through the upper portion showed its early growth to be flattened (fig. 8). The greater number of laterals per whorl (1920-1927) is probably indicative of a still earlier tendency toward fasciation. The laterals of the 1928 and 1929 whorls showed few signs of fasciation, while the 1930 and 1931 growths were very severely fasciated, with the 1931 growth showing the greatest amount and the most extreme examples of fasciation. TANNER (7) shows that normal cylindrical twigs may give rise to fasciated twigs, and on the other hand fasciated twigs may give rise to normal twigs. This is borne out in the present study.

The greatly weakened growth of the 1928 leader in 1930 and 1931 (fig. 9) indicates what effect the disease may have. Very likely the two 1931 leaders would also have become greatly weakened in their growth had they been allowed to continue growing. Continuation of such stunting effects, accompanied by extreme production of twigs and forking, would materially lessen the value of the tree for the production of lumber.

It will be of considerable physiological interest to observe whether one of the apparently normal laterals in the whorl, just below where the top of the tree was cut off when the specimens were gathered, will grow erect and exhibit fasciation in its new growth.

AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONN.

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COMPOSITION OF THE BEANS OF *PARKINSONIA ACULEATA*

ROBERT A. GREENE

Introduction

Parkinsonia aculeata, known also as the "Jerusalem thorn," and more commonly in the southwest as "palo verde" or "bagote," is common to all tropical countries. It is found in abundance in the southwest, where the tree is often cultivated for ornamental purposes, but also grows wild.

This species is very hardy and has a low water requirement. It is found on the bajados, canyons, and especially in the foothills near Tucson, where the average annual rainfall is about 12 inches. This plant shows a definite physiological response to environment. The leaves are composed of one to several long rachises and numerous minute leaflets, this adaptation materially reducing the transpiration losses. The chlorophyll and pigments associated with photosynthesis are found chiefly in the branches and stems, giving the tree a green appearance; hence the colloquial name "palo verde." Consequently practically all photosynthetic processes are carried on in the bark of the tree, rather than the leaves.

Parkinsonia aculeata is a legume, producing a considerable quantity of oblong seeds, contained in narrow pods 2-4 inches long. The seeds make up approximately 70 per cent of the weight of the whole bean. In Arizona these mature in the late summer, usually about the months of July and August.

In spite of the relative abundance of this plant, it has but little use at present, and apparently no studies of the chemical composition of the seed have ever been made. Apparently the only mention of the use of these seeds for feed by animals was made by Dr. R. H. FORBES of Tucson, who saw African monkeys strip the pods from the trees, break them open and eat the seeds. Usually the monkeys eat the seeds before they are fully ripe since the seed coat is not so hard at the time. On the range, live stock eat some of the small

tender twigs. FORBES and SKINNER (4) state: "palo verde twigs are an instance of browse forage upon which animals are so largely dependent in a semi arid country where grass is short."

Dr. J. J. THORNBURGH informed the writer that sometimes the Indians use the seeds as food, either cooked or raw. Since the seeds of leguminous plants contain considerable quantities of protein, this study was commenced in order to determine what possible food value the seeds might have.

Experimentation

The seeds were gathered on the campus of the University of Arizona, and since they were collected from a considerable number of trees, the sample should be representative.

TABLE I
FEEDING ANALYSIS (AIR-DRY BASIS)

	PERCENTAGE		
	PODS ONLY	WHOLE BEAN	SEEDS ONLY
Moisture.....	5.04	5.50	5.70
Ash.....	3.85	3.99	4.14
Protein.....	5.75	16.37	25.12
Fat.....	0.09	1.11	1.83
Crude fiber.....	50.38	27.94	19.84
Nitrogen-free extract.....	34.89	45.09	43.37

The seeds are contained in a narrow pod averaging about 3 inches in length. A quantity of the pods were threshed by placing them in a sack, beating with a small club, and then separating the pods from the seeds by winnowing. Both the pods and seeds were ground separately, and some of the "beans" were ground whole, without separating the pods and seeds.

The seeds are somewhat oblong in shape, have an extremely hard seed coat, and are very difficult to grind. On account of this hardness they must be soaked for four or five hours in concentrated sulphuric acid to promote germination. They may be exposed to the acid for a longer period of time without causing injury.

The pods, seeds, and the whole beans were finely ground, and the usual feeding analysis was made, using the methods of the Association of Official Agricultural Chemists (1). In order to secure

some idea of the carbohydrate composition of the pods, a proximate analysis was made, according to the methods of WAKSMAN and STEVENS (7). These results are given in tables I and II.

TABLE II
PROXIMATE ANALYSIS OF PODS
(MOISTURE-FREE BASIS)

	PERCENTAGE
Ether-soluble fraction.....	0.09
Cold water-soluble organic matter.....	6.17
Hot water-soluble organic matter.....	2.27
Alcohol-soluble fraction.....	1.49
Hemicelluloses.....	30.97
Celluloses.....	22.81
Lignins.....	19.56
Crude protein.....	4.92
Ash.....	4.05
Total.....	92.33

The seeds were treated with various peptizing agents, in order to determine the classes of proteins present. The method was similar to that employed by JOHNSON (6) in his study of tubercule bacilli. The results appear in table III.

TABLE III
DISTRIBUTION OF NITROGEN IN SEEDS

	PERCENTAGE TOTAL N
Albumin (water-soluble).....	18.41
Globulin (soluble in 10% NaCl).....	6.47
Glutelins (soluble in 0.2% NaOH).....	13.18
Prolamines (soluble in 70% C ₂ H ₅ OH).....	5.72
Other proteins (by difference).....	56.22

Discussion

Table I shows that the pods contain large amounts of crude fiber and nitrogen-free extract. A proximate analysis made by the method of WAKSMAN and STEVENS (7) showed that hemicelluloses, celluloses, and lignins make up about 73 per cent of the dry weight of the pods. Further tests indicated that the pods contain considerable quantities of pentosans. Possibly the pods might be used for the manufacture of paper pulp or for the preparation of furfural from the pentosans.

Since the market is at present flooded with readily available materials for these purposes, there seems to be no commercial possibilities for utilizing the pods.

The composition of the whole bean is similar to that of a good grade alfalfa hay (2, 5). The crude fiber content of the two are practically the same, which indicates that each should have the same relative digestibility. There is also a similarity in composition between the whole bean of this plant and those of mesquite (3).

The seeds contain a considerable amount of protein but only a small percentage of fat or oil. This quantity is entirely too small to have any commercial value. The amount of crude fiber is somewhat less than that of the whole bean, but in this case the determination is not a true index of digestibility, since the seeds had been ground. It is extremely doubtful whether an animal could crush the seeds, although if they were ground they could be used as an emergency forage. The fact that the seeds are sometimes used as human and animal food is evidence that cyanogenetic glucosides or other toxic substances are not present.

Table III shows that an albumin and glutelin are probably the principal proteins present. The results for globulins appear low, since they are usually the principal proteins present in seeds. In this case, however, the globulins were extracted by 10 per cent sodium chloride, which is usually employed for such purposes. The studies of JONES, JOHNS and coworkers at the United States Department of Agriculture show that 10 per cent sodium chloride is not always the best peptizing agent for globulins. It is probable that lower concentration might have peptized a large percentage of the globulin. It is planned to extend this study by isolating the proteins and to determine the various amino acids present.

Summary

1. The pods, whole beans, and seeds of *Parkinsonia aculeata* have been analyzed. Hemicelluloses, celluloses, and lignins compose approximately 73 per cent of the pods.
2. The composition of the whole bean is similar to that of alfalfa hay or mesquite beans.
3. The principal proteins of the seed are albumin and glutelin.

4. If proper means for grinding these beans or seeds are available, they should provide a good emergency forage.

The writer acknowledges his indebtedness to Dr. E. L. ANDERSON who collected the beans; and to Dr. J. J. THORNER who identified the species and offered many valuable suggestions.

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RELATION OF LENTICELS AND SURFACE AREA TO RESPIRATION IN THE POTATO TUBER

W. H. MICHAELS

That the permeability of the periderm of the potato tuber is a limiting factor in the exchange of gases between the tissues of the tuber and the surrounding air has been shown by a number of workers, of whom SMITH (3) is probably the most recent. So far as the writer is aware, no one has attempted to determine the relationship existing between the lenticels and the gaseous exchange of the tuber. The present paper reports such a study.

The work reported here was done at South Dakota State College during the winter of 1924-25, with potatoes which had been grown on the College farm the previous season. Clean, smooth, apparently disease-free tubers of the Irish cobbler, Early Ohio, and Green Mountain varieties were used.

The respiration rate was determined by the continuous aeration method, the respired carbon dioxide being absorbed in barium hydroxide. All determinations were carried out at room temperature, which remained nearly constant at 22° C. Determinations were made at 24-hour intervals for a period of seven days and were in duplicate.

To count the lenticels, the tubers were boiled and the entire skin removed, cut into pieces approximately 2 cm. square, placed on a moistened glass plate and held up to the light. The lenticels were then easily counted, after which the pieces of skin were laid on cross-section paper and their area estimated.

The data obtained are presented in table I. In general, the amount of carbon dioxide respired per kilogram of tubers increases from large to small tubers. The medium sized Green Mountain tubers are an exception, probably owing to the difficulty of selecting a truly median class of tubers. The relationship between surface area and size shows a similar trend. These results agree well with those of HOFFMAN (1), who came to the conclusion that the higher respiratory rate of the small tubers was due to their greater surface

area in proportion to volume. It will also be noted that the number of lenticels per kilogram increases from large to small tubers. There thus appears to be a relationship between the rate of respiration, surface area, and number of lenticels per kilogram of tubers.

TABLE I

MILLIGRAMS CARBON DIOXIDE RESPIRED AT 22° C.; SURFACE AREA AND
NUMBER OF LENTICELS FOR LARGE, MEDIUM, AND
SMALL POTATO TUBERS

SIZE	NUMBER OF TUBERS	CARBON DIOXIDE (MG.)			SURFACE AREA IN SQ. CM. PER KILO	LENTICELS	
		PER KILO PER HOUR	PER SQ. CM. PER HOUR	PER LEN- TICEL PER HOUR		PER KILO	PER SQ. CM.
Early Ohio							
Large.....	4	34.35	0.0296	0.0126	1162	2731	2.35
Medium.....	4	42.66	0.0312	0.0123	1368	3475	2.54
Small.....	6	58.02	0.0329	0.0075	1765	7713	4.37
Average.....		45.01	0.0314	0.0097	1432	4640	3.09
Irish Cobbler							
Large.....	4	15.75	0.0128	0.0061	1233	2564	2.08
Medium.....	4	20.69	0.0149	0.0053	1391	3895	2.80
Small.....	6	21.03	0.0124	0.0039	1702	5429	3.19
Average.....		19.16	0.0135	0.0049	1442	3963	2.69
Green Mountain							
Large.....	4	15.58	0.0118	0.0041	1321	3778	2.86
Medium.....	6	14.99	0.0116	0.0033	1266	4601	3.55
Small.....	6	17.52	0.0104	0.0032	1687	5466	3.24
Average.....		16.03	0.0112	0.0035	1435	4615	3.22

Carrying the analysis somewhat further, it is seen from table I that the amount of carbon dioxide respired per square centimeter of surface shows no consistent differences between large and small tubers, despite the much larger volume of tissue dependent upon a unit of surface for gas exchange with the surrounding air in the case of the large tubers. The amount of carbon dioxide respired per lenticel, however, decreases from large to small tubers. This is no doubt due to the higher number of lenticels per unit of surface in the

small tubers, as is shown in the last column of the table. It is also probable that the lenticels of the large tubers have a somewhat greater diameter than those of the small tubers.

These observations indicate that the lenticels are the chief avenue of gas exchange between the internal tissues of the potato tuber and the surrounding air. The work of SMITH has shown that the internal tissues offer considerable resistance to the rapid movement of gases into or out of the tuber, which probably largely accounts for the low rate of gas exchange in the large tubers. It hardly seems probable that the smaller number of lenticels per unit of area on the large tubers could be the factor slowing down the respiration rate in the large tubers, since LUTMAN (2) has shown that the lenticels are capable of accommodating a higher rate of gas exchange than normally occurs. He cut tubers in half, hollowed out the halves, tied them together and sealed the union with vaseline. Owing to wound response these tubers respired at a much higher rate than the normal checks; yet the gas exchange was all through the periderm which had not been injured except at the point of the union sealed by vaseline.

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RELATION OF PINOXYLON DAKOTENSE KNOWLTON TO PROTOPICEOXYLON GOTHAN

RICHARD KRÄUSEL

A redescription of *Pinoxylon dakotense* Knowlton, a coniferous wood of the Lakota sandstone (Lower Cretaceous, Black Hills), has been given by READ (6). He finds that it is no *Pinus* but belongs to *Protopiceoxylon* Gothan. Thus he confirms the supposition already made by me in 1919. It is surprising that READ does not make mention of my paper (1), as it affords a critical list of all the fossil coniferous woods (with exception of *Araucarioxylon* Kr.) known up to that time. As to *Pinoxylon dakotense*, I wrote, "Dieses angeblich zu *Pinus* gehörende Holz steht in enger Beziehung zu *Protopiceoxylon*."

In that same paper, one might have found that the description of *Protopiceoxylon salisburioides* (Göpp.) Kr. is by no means "brief and without illustrations" (READ). READ refers only to my preliminary statement of 1913 (2), overlooking the full and richly illustrated account given in 1920 (3). There it is shown that the peculiar anatomical features of that wood from the Miocene lignite (browncoal) (for example, resin ducts, strongly pitted walls of the rays, etc.) are traumatic, and that the wood is of true abietinean affinity. Therefore it was excluded from Mesozoic *Protopiceoxylon* species and the name *Cedroxylon salisburioides* was proposed.

May I mention that twice (1, 4) I considered the bearing of wood anatomy and of fossil woods on the phylogeny of conifers? I came to the conclusion that JEFFREY was wrong and that the Araucarieae are older than the Abietineae. But we both were wrong. Abietineae and Araucarieae are groups of living (and extinct) plants, characterized by distinct anatomical and morphological features. If we find a Mesozoic or Paleozoic conifer with araucarian wood but abietinean cones, or vice versa (and I think they will be found one day), it would be wrong to include them in the Abietineae (or Araucarieae). They form, on the contrary, a new, extinct group of conifers, from which

both the living Araucarieae and Abietineae might have descended. Since only the anatomical side of the problem is known, we are not justified in speaking (as JEFFREY and I and other writers have) of the age of Abietineae and Araucarieae, but only of the age of the one or other type of wood. And here we may state that among fossil conifers the araucarian type of wood is the older one. It should not be forgotten, however, that the Mesoxyleae of the Paleozoic are in no way connected with true conifers. But even among them we find *Abietopitys*, a form with "abietinean" pitting of the rays (5).

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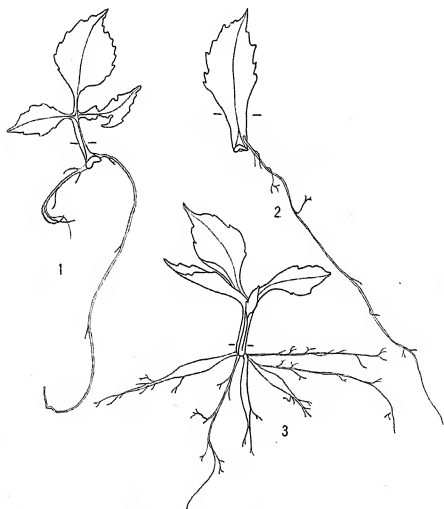
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BRIEFER ARTICLE

ROOT FORMATION FROM LEAF CUTTINGS

While experimenting with various kinds of leaves and portions of leaves which might be used as cuttings at different times during the year,



FIGS. 1-3.—Fig. 1, leaf cutting put in soil October 2; diagram made November 24; fig. 2, segment cutting put in soil October 2; diagram made November 24; fig. 3, leaf cutting showing tuber-like roots.

well-rooted cuttings have been secured by using single leaves of *Dahlia variabilis* and imbedding 20 mm. of the petioles in sand (fig. 1). Roots have also been developed at the same time of year, using only the termi-

nal segment (fig. 2). The cuttings rooted in eight weeks, and about two weeks later the roots were beginning to thicken (fig. 3). The plants developed and blossomed the next summer. Since it is necessary to take only a few leaves from the plant late in the fall to start an equal number of plants, this method does not greatly interfere with the storage of food material in the root.

It is hoped to try this experiment at different times during the year, and at the same time to analyze the food content, together with the internal structure of both the petiole and the base of the terminal segment of the leaf.—EDITH A. ROBERTS AND JULIA R. LAWRENCE, *Department of Botany, Vassar College, Poughkeepsie, New York.*

CURRENT LITERATURE

Announcement

Under the new arrangement for quarterly publication the Botanical Gazette can use two or three longer papers in each issue, such papers being accepted at the discretion of the editors. This plan removes the ruling regarding page limits for manuscripts.

Papers being accepted as this note goes to press will appear in the coming June issue.

BOOK REVIEWS

Chemistry and technology of oils and fats

The second edition of UBBELOHDE'S *Handbuch der Chemie und Technologie der Öle und Fette* appears in four volumes (two of which are in two volumes each, making six volumes), under the editorship of Dr. H. HELLER of Magdeburg. The first volume (pp. xvi+791) discusses the chemistry and technology of the oils, fats, and waxes. The second volume (pp. xii+824) is divided into two parts, the first of which deals with the chemistry and technology of vegetable oils and fats. The second part is still unpublished. Volume III, also in two parts, treats in part 1 (pp. xx+392) the chemistry, analysis, and technology of the fatty acids, glycerol, and Turkey-red oils; and in part 2 (pp. xx+752) the chemistry and technology of soaps and washing materials. The fourth volume (pp. xiv+798) is devoted to the oxidized, polymerized, and reduced fatty acids, and the waxes.

This review will consider only the first part of volume II,¹ on the vegetable oils and fats, which has just been published. The work falls into four sections, as follows: strong drying oils; weak drying oils; non-drying oils; and the solid vegetable fats. In treating these materials, the arrangement is based on the plant families, passing in order from the lowest to the highest families in which the given type of oil is found. The section on strong drying oils begins with those found in the Taxaceae and Pinaceae, and ends with those of the Compositae, such as burdock seed oil, thistle oil, sunflower oil, dandelion oil, cocklebur oil, etc. Similarly the section on weak drying oils begins with those of the Gramineae (corn oil), proceeds to the oils of such families as the Ranunculaceae, Cruciferae, Leguminosae, Euphorbiaceae, Malvaceae, Umbelliferae, etc., and

¹ HELLER, HANS. UBBELOHDE'S *Handbuch der Chemie und Technologie der Öle und Fette*. Vol. II, part 1. *Chemie und Technologie der pflanzlichen Öle und Fette*. 8vo. pp. xii+824. Hirzel. Leipzig. 1932.

ends with weak drying oils of such composites as artichoke, lettuce, and golden-rod. The sections on non-drying oils and solid fats begin with those of the fungi.

The method of arrangement gives the work an encyclopedic character; it is an excellent dictionary of the vegetable oils and fats. The table of contents lists the page references in four columns, headed by the names of the four types of vegetable oils and fats, and the first column lists the names of the plant subkingdoms and families from *Thallophyta* to the highest family in the *ENGLE* and *PRANTL* system. The *Bryophyta* alone have no representatives in the list.

There is a brief appendix giving the results of a few papers published from 1929 to 1931 that were not seen in time to include in the main text. The work of almost 1500 investigators has been drawn upon for the data included in this part I of volume II. It is on the whole a very valuable summary, and will be indispensable to those who wish information on the distribution of the vegetable oils and fats in nature. It will also be a source of information as to the characteristics of the oils. In tabulated form it presents such information as is obtainable as to the specific gravity, melting points, solidification points, refractive indices, acid numbers, saponification numbers, iodine numbers, Reichert-Meissl numbers, acetyl values, and percentage of unsaponifiable fats, for the oils and fats listed. One can therefore obtain in very concise form whatever is known concerning almost any vegetable oil or fat that is mentioned in the literature.

—C. A. SHULL.

A taxonomic study in *Potamogeton*

In the past, misdeterminations of American species of *Potamogeton* have been almost as common as the mispronunciation of the generic name. A recent notable work by FERNALD² should do much to clarify our knowledge of these species and to render a proper identification reasonably certain. For some fifteen years FERNALD has studied, although rather intermittently, the genus in a monographic way, his investigations including the first-hand examination of "approximately 12,000 sheets." It is cause for rejoicing among all who have the welfare of taxonomic conservatism at heart when an authority of such exacting scholarship devotes his best thought and effort to the revision of such a group. Especially is this true of *Potamogeton*, "for, unfortunately," as we find stated in quite another connection, "the group has been so generally avoided by botanists of accurate minds and scholarly temperaments, that it has in recent years drifted largely into other hands and has been treated in an impressionistic and slipshod manner almost unbelievable."

² FERNALD, M. L., The linear-leaved North American species of *Potamogeton*, section *Axillares*. 4to. pp. 183. Pls. 40, maps 31. *Memoirs American Academy of Arts and Sciences*. Vol. XVII. part I. 1932; reprinted in *Memoirs of the Gray Herbarium of Harvard University*, no. III. 1932.

The text falls into five major divisions, entitled: introduction; diagnostic characters (fruits, spikes, sepaloïd connectives, peduncles, stipules, leaves, nodal glands, winter buds or hibernacula, stems, and rhizomes); geographic distribution of the subsections and species (broad distribution of the subsections, interrupted ranges in North America); synoptic treatment of the North American species (material studied, illustrations, maps, the classification of North American pondweeds, key to the indigenous linear-leaved North American species of *Potamogeton* § Axillares, synopsis of species); and list of exsiccatae cited. The introduction gives an extended historical survey with many pointed criticisms and observations. The main body of the text abounds in distributional, geological, and other types of data and the individual taxonomic treatments are supplemented with numerous extended discussions that greatly enhance their value. The geographic range of various species and varieties is shown graphically by dotted maps. Forty full-page plates of half-tone illustrations serve to give additional guidance in the interpretation of the text. Generally speaking, these represent a high standard of excellence. The "list of exsiccatae cited" is extensive but does not include, as one might infer from the title, the numerous unnumbered specimens cited in the text. Occasional slips occur, but fortunately these are not abundant.

FERNALD's memoir is characterized throughout by an attempt to cut loose from "the impressionistic," the subjective, one might even say "the psychological" bases for delimiting species and subspecific entities. It represents a commendable effort to associate taxonomic units with tangible morphological characters; in short, to produce a treatment resting upon a firm objective foundation.—E. E. SHERFF.

Manual of bryology

A volume has just been published under the editorship of FR. VERDOORN,³ in collaboration with fifteen other contributors. It represents an effort to bring together in one volume the most recent research on the Bryophyta in several fields of investigation. The first two chapters deal with the morphology and anatomy of the mosses and liverworts respectively, the former being treated by R. VAN DER WIJK and the latter by H. BUCH. The treatment in these chapters is somewhat general but adequate for a volume of this type. It is to be regretted that these sections are not more completely illustrated. The present status of experimental morphology is treated by BUCH in chapter III. The fourth chapter deals with germination of spores and development of protonema, presented by G. CHALAUD.

The ecological aspects of the Bryophyta are treated in several separate chapters, which, curiously enough, are not arranged in sequence. Chapter V, on the

³ VERDOORN, FR. (editor) et al., *Manual of bryology*. pp. viii+485. 129 illus. Martinus Nijhoff. The Hague. 1932.

association of bryophytes with other organisms, is presented by G. NICHOLAS; chapter X, covering geographical distribution, is written by TH. HERZOG; and quaternary distribution is treated by H. GAMS. Other ecological phases dealing with moss societies are presented in chapter XII by the same author, with a concluding chapter on ecology by P. W. RICHARDS.

The cytological and genetical aspects of the bryophytes are very ably handled in chapter VI on cytology, by J. MOTTE; chapter VII, dealing with nuclear phases, by K. HOEFER; and chapter IX on genetics, by F. VON WETTSTEIN. The section devoted to physiology was prepared by A. J. M. GARJEANNE. The concluding chapters deal with the taxonomy and phylogeny of the bryophytes. The classification of the mosses is presented by A. N. DIXON, that of the hepatics by FR. VERDOORN, while the phylogenetic aspects are presented by W. ZIMMERMANN.

While it is obviously impossible to treat such a wide array of subjects adequately within the scope of a single volume, it is nevertheless encouraging that a manual of this type has been attempted. It will serve as a valuable handbook for all those interested in bryology; and since there are adequate bibliographies or reviews of the significant literature under each section, it will be very helpful to workers in this field in making contacts with original sources.—H. E. HAYWARD.

College textbook of botany

MOTTIER has revised and amplified his *College textbook of botany for first-year students*, so that it now appears as a *Textbook of botany for college students*.⁴

Structure, function, and life history are described in concrete form, while touches of human interest brighten the presentation. The work begins with the life history of a seed-bearing plant, following which there is a chapter on the cell. The author's long series of researches on cell structures, dating from the nineties, when he was studying with STRASBURGER, have enabled him to write a much more authoritative account than could be expected from textbook writers whose first-hand knowledge of cell structures is limited or even lacking altogether. In succeeding chapters, leaf, stem, and root are described in detail, the structure and function being correlated throughout. In addition, there is a special chapter on the movement of water and food in plants. Seeds and seedlings, utilization of food, energy release, and growth and response to stimuli are the subjects of several succeeding chapters.

The foregoing features occupy 185 pages; the following 342 pages are devoted to plants arranged in taxonomic sequence from the myxomycetes to the angiosperms. The book closes with chapters on the dispersal of seeds and fruits, geographical distribution of plants, and heredity.

⁴ MOTTIER, D. M., *A textbook of botany for college students*. 8vo. pp. x+601. Figs. 542. P. Blakiston's Son and Co. Philadelphia. 1932.

From the standpoint of the reviewer, the treatment of alternation of generations is inadequate, for he believes that wherever there is sexuality there must be an alternation of generations. In an elementary book this subject might properly begin with the bryophytes; but, in an advanced college text, the origin and evolution of alternation should be considered in the thallophytes since groups above this level illustrate only the evolution of an already well established sporophyte and the reduction of the gametophyte. We all use the term sporophyte and gametophyte; but the fact that they are misnomers in many of the thallophytes indicates that they do not correspond to fundamentals in the phenomenon of alternation.

The presentation throughout is what would be expected from a teacher who has long been an active investigator. There are 542 illustrations. The author draws so well and so accurately that the extensive borrowing of illustrations, a few of which are incorrectly credited, seems unfortunate. The originals, especially in pteridophytes and spermatophytes, are excellent.

At the end of each chapter there are stimulating topics for review and discussion. The whole presentation is so practical and has such an atmosphere of the laboratory about it, that no specific laboratory directions are necessary.—C. J. CHAMBERLAIN.

Handbook of plant analysis

The first of KLEIN's volumes on plant analysis was noted in this journal⁵ some months ago. The second volume has now appeared,⁶ and it is evident that the work as a whole is to be a most useful compilation of methods. This volume takes up the analysis of plant tissues for inorganic and organic constituents. The first section outlines the methods for determination of the inorganic substances in four groups: (1) the most important cations and anions, and occasionally occurring elements; (2) inorganic nitrogenous ions; (3) preparation and analysis of plant ash; and (4) gas and micro-gas analysis.

The section on organic substances covers the most important of the organic plant constituents. The subsection headings are as follows: alcohols; aldehydes and ketones; phenols and quinones; organic acids; phosphoric acid esters of carbohydrate metabolism; general consideration of "lipoids"; fats and waxes; phosphatides; phytosterols; sugar alcohols (polyhydric); simple carbohydrates (mono- and di-saccharides); characteristics, preparation, and determination of individual sugars; and polysaccharides (inulin, glycogen, starch).

At the close of each chapter a list of plant families is given, in which each of the substances treated occurs. This statement of the distribution of compounds should be helpful. While it is very difficult to make such lists even tolerably complete, one can obtain a fair idea of the distribution of certain constituents by

⁵ BOT. GAZ. 92:332-333. 1931.

⁶ KLEIN, G. *Handbuch der Pflanzenanalyse*. Vol. II. 8vo. pp. xii+973. Julius Springer. Vienna. 1932.

consulting these condensed statements. Occasionally very erroneous statements are made regarding the distribution of substances in nature. For instance, the statement has recently been made⁷ that inulin had been found thus far only in two plant families. HAAS and HILL, however, mention a dozen or more families, and KLEIN's *Handbuch* mentions more than twenty families in which inulin has been found, not counting the ferns, fungi, and algae.

Among the contributors who have made this excellent work possible are men who have contributed largely to our knowledge of the substances they treat. RIPPEL, PRIANISCHNIKOW, NEUBERG, WEHMER, WINTERSTEIN, and PRINGSHEIM are among those who have worked with KLEIN. One has confidence in a work which is produced by the cooperative efforts of expert plant chemists. The completed work is to consist of four volumes. The remaining ones will be awaited with much interest.—C. A. SHULL.

Microchemistry

A translation of EMICH's manual of microchemistry has been made by SCHNEIDER.⁸ The book in the German passed through two editions. The English edition is brought up to date by the inclusion of material published since the second German edition went to press. Also, there are included references to American literature and sources of supply of apparatus.

The book is divided into two main parts. The first considers apparatus and methods, and the second describes practice exercises. Each of these parts is further divided into a qualitative section and a quantitative section. Some of the subjects discussed in the first part are: the microscope, including the polarizing microscope; reagents; treatment of precipitates; recrystallization; boiling point determination and fractionation; sublimation; schlieren observations; qualitative microanalysis with small electrodes; and mounting permanent microchemical preparations. The second part of the book, including the section of spot analysis, describes 84 exercises, applying the methods described in a general way in the first part. These exercises have mainly to do with the detection and the quantitative determination of the amount of various ions and compounds. There is an inorganic section and an organic section. There are also exercises on the use of the microscope, including the polarizing microscope.

The book is mainly of value to the chemist, and he should find it of very great help. The author has not tried to include botanical microchemistry, such as given in the standard textbooks of TUNMANN and MOLISCH. Yet botanists, especially plant physiologists, will find certain sections of great value, especially

⁷ Amer. Jour. Bot. 19:187. 1932.

⁸ EMICH, FRIEDRICH, Microchemical laboratory manual, with a section on spot analysis by FRITZ FEIGL. Trans. by F. SCHNEIDER. pp. xvi+180. Figs. 88. John Wiley and Sons. New York. 1932.

those having to do with the microscope and the polarizing microscope, reagents, sublimation, and some of the qualitative exercises on the inorganic cations and anions. But there is still great need of a textbook in English on botanical microchemistry.—S. V. EATON.

Tropical woods

A publication⁹ which is issued four times a year by the Yale School of Forestry, for the subscription price of \$1.00, contains very interesting articles dealing with anatomy, morphology, taxonomy, and ecology of tropical woods, giving proper attention to their usefulness. It includes not only original articles but also book reviews on important literature pertaining to tropical silviculture. Up to now twenty-nine numbers have appeared, of which the last one is for March 1, 1932. The wealth of information which this magazine contains may be judged from a few titles taken from recent issues.

KANEHIRA, RYOZO, Forest trees of Micronesia.

DESCH, H. E., Significance of numerical values for cell dimensions.

CHATTAWAY, M. N., Proposed standards for numerical values used in describing woods.

RECORD, SAMUEL J., Brazilian kingwood (*Dalbergia cearensis*).

STANDLEY, PAUL C., Mexican and Central American species of *Pterocarpus*.

———, Two new trees from South America.

RECORD, SAMUEL J., Notes on Brazilian timbers.

PTITTIER, H., Note on *Escallonia tortuosa* H.B.K.

WILLIAMS, L., Forests of northeastern Peru.

MOLDENKE, HAROLD N., Three new species of *Aegiphila* from Central America.

DAHLGREN, B. E., Tupi manner of forming names of trees.

MACBRIDE, J. FRANCIS, South American *Viburnums* incorrectly described as new species of *Cornus*.

AITKEN, J. B., Wallabas of British Guiana.

COOPER, G. PROCTOR, Expavé (*Anacardium rhinocarpus* DC).

EVES, LUIS J., Apitong (*Dipterocarpus* spp.) of Northern Negros.

HÉDIN, L., Commercial mahoganies of French Cameroon.

RECORD, SAMUEL J., Notes on Australian woods.—A. C. NOÉ.

Waste products of agriculture

A volume of considerable interest to the practical botanist and agriculturist has been written by HOWARD and WAD.¹⁰ The book is concerned with the problem of the utilization of the by-products of the farm in increasing the fertility of the land. The authors are well equipped to discuss such a subject, for they are

⁹ Tropical woods, edited by SAMUEL J. RECORD, School of Forestry, Yale University.

¹⁰ HOWARD, ALBERT, and WAD, Y. D., The waste products of agriculture and their utilization as humus. pp. ix+167. Oxford University Press. 1931.

thoroughly familiar with the intensive systems of agriculture of the Orient. It is only there that much has been done in making use of the wastes of the farm. The senior author has been studying the problems of crop production in India for 26 years.

The main topics discussed are: a comparison of the agricultural systems of the Occident and the Orient; the sources of organic matter and its relation to soil fertility; the Indore process for the manufacture of humus, with the main factors involved and the practicability of using the process in other areas.

The book should do much to stimulate interest in the more complete and efficient utilization of the by-products of the farm in the United States and other countries where the extensive system of agriculture is used. One of the main difficulties in the way of adopting the Indore process in these countries is, of course, the scarcity of labor. But, as pointed out by the authors, mechanization should solve this problem. Another difficulty would probably be the opposition of the manufacturers of artificial fertilizers. But if one or two agricultural experiment stations would demonstrate the value of the process in increasing crop yields, there might be a gradual adoption of the plan in spite of this opposition. This would not mean that artificial fertilizers would not be used, for the best results are obtained when the humus and artificial materials are combined.—S. V. EATON.

Megaspores from coal seams

In recent years very important studies have been made of the spores found in some German and Polish coal beds.¹¹ The method which has been successfully introduced for the preparation of pollen diagrams in peat bogs has now been used for coal seams, and a great many different types of megaspores have been recorded for various seams and at various horizons in the seams. It is very difficult to determine to what species of fossil lycopods or Equisetales these spores belong, and therefore it is necessary to establish form types of these spores either

¹¹ ZERNDT, J., *Megasporen aus einem Flöz in Libiaz (Stéphanien)*. Extrait du Bulletin de l'Académie Polonaise des Sciences et des Lettres. Classe des Sciences Mathématiques et Naturelles. Série B: Sciences Naturelles (1). 8vo. pp. 39-70. Pls. 1-8. Cracow. 1930.

———, *Triletes giganteus*, n. sp., eine riesige Megaspore aus dem Karbon. Extrait du Bulletin de l'Académie Polonaise des Sciences et des Lettres. *Ibid.* pp. 71-79. Pls. 9-11. Cracow. 1930.

———, *Megasporen als Leitfossilien des produktiven Karbons*. Extrait du Bulletin de l'Académie Polonaise des Sciences et des Lettres. Classe des Sciences Mathématiques et Naturelles. Série A: Sciences Mathématiques. 8vo. pp. 165-183. Pls. 3-10. Cracow. 1931.

POTONIE, R., *Sporenformen aus den Flözen Ägir und Bismarck des Ruhrgebietes*. Neues Jahrbuch für Mineralogie etc. Beil. 67: Abt. B. 8 vo. pp. 438-454. Pls. 14-20. Berlin. 1932.

under the genus names *Triletes* or *Sporonites*. It is hoped that gradually it may be possible to identify the relation of spores to known plant species. Meanwhile a large and desirable amount of information has been made available about the spores themselves.—A. C. NOÉ.

Physical chemistry of sexuality

JOYET-LAVERGNE has written a significant book¹² on the physical chemistry of sexuality, the volume being one of the *Protoplasma* monographs. It is a general treatise on the subject, including both plant and animal work. The nature of the material of the book is indicated by reference to the titles of some of its 14 chapters. There are chapters on various sexual differences, morphological, physiological, and physico-chemical. Other chapters treat the genital hormones, the chemical differences characteristic of sex, cytoplasmic sexuality, intersexuality, the change of sex, the physico-chemical conception of sexuality, various theories on the problem of sexuality, intersexuality, and metabolism in relation to sexuality. In the latter chapter the work of RIDDLE on the metabolism of the pigeon is discussed. The book gives a critical, unbiased discussion of the whole field of sexuality. There is a bibliography of about 50 pages, containing hundreds of references to American and English work. The volume will be welcomed by the botanist and zoologist, especially those interested in genetics.—S. V. EATON.

Vegetation of the Canary Islands

The position of the Canary Islands, so near the coast of Africa and yet so different from it in vegetation, has for many decades made their flora of special interest to botanists. By their Tertiary relicts and their numerous endemics these islands seem almost to belong to another world. Interest shown concerning the vegetation in past years will be greatly augmented by an elaborate treatise on the ecology and distribution of the elements of the flora which has recently appeared.¹³ The author has discussed in detail the age, the geology, and the climate of the group, together with the influence of these factors on the flora as a whole. Each of the seven islands is then considered individually with respect to the peculiarities of its flora, and finally the individual species are discussed and illustrated. The large size of the volume (9×12 inches) permits the reproduction of the numerous photographs in elaborate and unusually fine plates. The work has been so carefully done, the descriptions are so thoroughly organized, and the illustrations are reproduced in such excellent plates that a graphic picture of a unique flora has resulted.—G. D. FULLER.

¹² JOYET-LAVERGNE, PH., *La physico-chimie de la sexualité*. 8vo. pp. xi+457. Gebrüder Borntraeger. Berlin. 1931.

¹³ BURCHARD, OSCAR, *Beiträge zur Ökologie und Biologie der Kanarenpflanzen*. *Bibliotheca Botanica* 98:1-262. Pls. 78; map. Erwin Nägele. Stuttgart. 1929.

Principles of soil microbiology

The excellent text by WAKSMAN¹⁴ dealing with the principles of soil microbiology has been extensively rearranged and revised. The new discussion on mycorrhizal fungi is valuable. Of especial interest to plant physiologists are the new chapters on the rôle of microorganisms in the decomposition of plant residues and the formation of peat. The discussion of the chemical dynamics of the soil is well done. This book is of considerable size (894 pages), and is an exhaustive treatment of the subject.—R. B. HARVEY.

¹⁴ WAKSMAN, S. A., Principles of soil microbiology. 2d ed. 8vo. pp. xxviii+894. Williams and Wilkins Co. 1932.

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EMBRYO ABORTION IN EARLY-RIPENING VARIETIES OF PRUNUS AVIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 439

H. B. TUKEY

(WITH FORTY-ONE FIGURES)

Introduction

The failure of early-ripening varieties of stone fruits, mainly of varieties of *Prunus*, to mature viable seed has been noted by both nurserymen and plant breeders. Nurserymen fail to secure good stands of seedling understocks of peach, mazzard cherry (*Prunus avium* L.), and mahaleb cherry (*P. mahaleb* L.) when the seed supply is obtained from wild trees which produce early-ripening fruit. Plant breeders find that crosses in which an early-ripening variety is used as the female parent produce viable seed only rarely.

BLAKE (1) pointed out that the flesh of some varieties of peaches ripens before the seed matures. MARSHALL (12) divided varieties of plum (*P. domestica* L.) into two groups, namely, those bearing mature fruits each of which contains a viable seed and those bearing mature fruits only a few of which contain viable seed. CHANDLER (3) reported never finding kernels in such early varieties of peach as the Sneed, although pollination is necessary for fruit production. CONNORS (4) stated that varieties of peaches ripening their fruit 75-80 days after pollination do not develop viable seed in New Jersey; that those which ripen 85-90 days after full bloom develop 15 per cent viable seed; and that later-ripening varieties produce a good

proportion of viable seeds. WELLINGTON (21) reported that records of cherry crosses indicated that early-ripening varieties used as parents had few viable seeds, medium-ripening sorts a higher percentage, and very late-ripening kinds a still higher percentage. TUKEY (18) showed that with 17 varieties and types of sweet cherry and 11 varieties and types of sour cherry, those kinds that were late-ripening (that is, requiring 80 days from full bloom to fruit ripening) developed nearly 100 per cent viable seed; those that were early-ripening (requiring 60 days or less from flower bloom to fruit ripening) developed almost no viable seed; and the mid-season sorts (those requiring 65-75 days to mature fruit) developed an intermediate number. SAKAGUCHI (17) recommends that plant breeders do not use as female parents peach varieties which ripen the fruit in fewer than 100 days after full bloom.

The problem presented by these facts suggests a critical comparison of the embryogeny and fruit development of early and late-ripening varieties of sweet cherry (*P. avium*). Furthermore, although critical studies have been made of both the early and the late stages in seed and fruit development, there is a dearth of material for the stages intervening. This paper attempts to supply information regarding these two phases of the subject.

Various phases in the embryogeny of the cherry have been reported by BRADBURY (2), MALPIGHI (11), PÉCHOUTRE (15), RUEHLE (16), TULASNE (20), WENT (22), and others. Studies in related genera which show similarity in development of related parts have been made by DORSEY (6) and OSTERWALDER (14). The growth of fruits of related species of *Prunus* has been studied by BLAKE (1), CONNORS (4), DORSEY and McMUNN (7), LILLELAND (10), and SAKAGUCHI (17). A more detailed mention of these findings will be made with reference to the particular point involved.

Procedure

Fresh material was dissected under the binocular microscope. This method provided a rapid means of securing an adequate number of measurements of the various stages of development. In addition, it proved useful in helping to interpret the prepared slides.

Flower and fruit parts from twigs placed in water and held at

2°C. for 72 hours showed no measurable development, thus permitting the gross examination of a great number of individuals over a comparatively long period of time at identical stages of arrested development.

Material from open-pollinated blossoms in a mixed varietal orchard was most satisfactory for general study because of its more uniform rate of development. For comparison with the open-pollinated blossoms, however, blossoms were emasculated before they had opened and were pollinated by hand 3 days later, when the stigmas were receptive. Pollen was used which had been removed in the laboratory from closed blossoms and ripened at a temperature of 25°–30°C. Emasculated blossoms were kept covered with tight paper bags from the time of emasculation until the fruit had set. The paper bags were then replaced by cheese-cloth bags. Open-pollinated, self-pollinated, and cross-pollinated material was prepared, the crosses being between known compatible varieties and the pollen being tested by germination at room temperature (20°–25° C.) in 5 per cent sucrose solution.

Pollen tubes were traced in the carpel under the dissecting microscope from fresh material crushed in lacmoid-martius-yellow after the method described by NEBEL (13). The varieties used for critical study were Early Purple Guigne, Burbank, Lyons, and Black Tartarian, representing early-ripening kinds; Eagle, representing a mid-season sort; and Windsor, Lambert, Downer, and Mazzard, representing late-season varieties. Others examined for comparison were Governor Wood, Knight's Early Black, Elton, Napoleon, Schmidt, Black Republican, Abundance, and Oswego. The work was conducted at Geneva, New York, throughout the growing seasons of 1930, 1931, and 1932. Records for the years 1926 to 1929 inclusive have been used to corroborate the findings from the more exact studies of 1930 to 1932 inclusive.

Material for slides made after the paraffin method was killed and fixed in two solutions, designated solution I and solution II. Solution I was made up of 100 cc. of 50 per cent alcohol, 2.3 cc. glacial acetic acid, and 6.7 cc. formalin; solution II (Karpechenko's chromacetic) was made up of two parts, part A consisting of 1 per cent chromic acid in water and part B consisting of 10 cc. of glacial acetic

acid and 40 cc. of formalin. The two solutions were kept separate until used for fixation, when two parts of A by volume and one part of B were poured together. The material was first placed in solution I while being prepared, then placed in solution II, the whole being then exhausted to 600 mm. of mercury in a desiccator, and held at 4° C. for 24 hours. Excellent fixation was secured by this method, the material being equally as good for cytological as for histological studies. In the case of ovaries 6-12 mm. in length, longitudinal slices were cut from both sides of the ovary, parallel to the sutures and including portions of the nucellus, thus permitting rapid penetration. With larger material the seed was removed from the carpel.

Safranin, orange G, and crystal violet were used for staining.

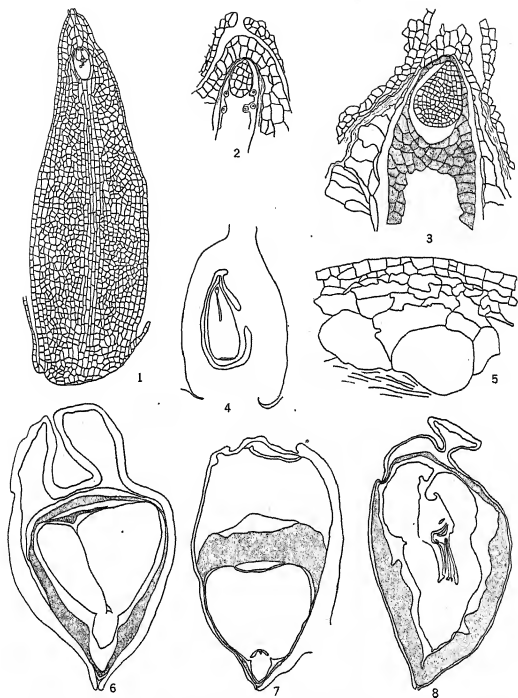
Measurements were made with an eyepiece micrometer, both sliding and fixed, and checked against each other. Care was necessary to secure median measurements and to secure fruits of average development. In the compilation of data shown in the tables, the mean of from three to seventeen individuals is represented for each measurement. The development of individuals collected at the same time showed very little variation, so that a smaller number would have served equally as well. Measurements from prepared slides were checked against those from fresh material dissected under the binocular microscope.

Normal development

Although certain stages and periods of development of particular parts of the cherry seed and fruit have been studied by other workers, no systematic record of development has been made, nor has any attempt been made to correlate the development of various parts. Accordingly the steps are here presented, and for this purpose the Downer variety of sweet cherry has been selected as typical or "normal." It is a late-ripening sort which normally produces a high proportion of viable seed.

1. OVARY.—The ovary increases in length only slightly from the 55th to the 14th day before full bloom, namely, from 0.54 mm. to 0.74 mm. At this time it begins a more rapid period of enlargement, until at the time of full bloom it is 2 mm. in length.

At the time of fertilization, a day or two after full bloom, there is



FIGS. 1-8.—Fig. 1, nucellus and 7-celled megagametophyte 2 days before full bloom, showing central core of conductive tissue of elongated cells from chalaza to megagametophyte through which the megagametophyte and embryo sac develop. Fig. 2, young embryo and free-nuclear endosperm 5 days after full bloom. Fig. 3, young embryo and cellular development of endosperm 12 days after full bloom. Fig. 4, development of megagametophyte, nucellus and integuments, and ovary from an emasculated, unpollinated blossom, 7 days after full bloom. Fig. 5, endosperm layer at chalazal end of seed, 37 days after bloom, showing growth by cell divisions in peripheral layer, region of cell enlargement, and region of digestion by adjacent advancing embryo. Figs. 6, 7, typical aborted seeds of Early Purple Guigne, 27 days after full bloom, showing peculiar flattening of cotyledons. Fig. 8, unusual embryo development in abortive seed, bearing 6 true leaves.

a sudden acceleration in the development of the pericarp, which continues for a period of 17 days after full bloom. During the season of 1931 the ovary increased in length from 2.46 mm. the 1st day after bloom to 3.31 mm. on the 4th, 4.38 mm. on the 6th, 7.5 mm. on the 13th, and to 10.5 mm. on the 17th. This is in contrast to the small increase prior to the time of fertilization. At the end of this period, development of the pericarp ceases almost as abruptly as it began, and a period of greatly lessened growth ensues, which in 1931 extended from the 17th to the 31st day, or a total of 14 days. A second period of rapid pericarp development follows the period of retarded development, continuing until the fruit is dead ripe, or from the 31st to the 66th day after full bloom during the season of 1931. Similar periods of accelerated and retarded pericarp development have been reported by BLAKE (1) and by DORSEY and McMUNN (7) for the peach, and by LILLELAND (10) for the apricot (*Prunus armeniaca* L.).

2. NUCELLUS AND INTEGUMENTS.—There are two ovules, arising from opposite placentae of the single carpel, only one of which develops to maturity. The micropyle points toward the distal end of the ovary and the micropylar end of the ovule lies against an outgrowth of the placenta, the obturator (RUEHLE 16). Both ovules develop equally but slowly up to 14 days before full bloom, paralleling the development of the ovary. They then begin a more rapid enlargement and double in length by full bloom unless arrested in development.

At the central axis of the nucellus, reaching from the chalaza to the base of the megaspore mother cell or of the megagametophyte, is found a core of elongated cells as shown in figure 1, which take gentian violet stain readily and split apart readily in a longitudinal direction. The surrounding cells of the nucellus are more nearly isodiametric, thin-walled, with less dense cytoplasm. The chalazal region is clearly marked as a deeply staining tissue.

Two to 7 days before full bloom one of the ovules is abruptly arrested in development, as mentioned by RUEHLE, but does not shrivel and collapse until 3 or 4 days after full bloom. In some varieties, as Lyons, it is common for both ovules to develop until the period of embryo abortion.

At the time of fertilization there is a sudden acceleration in growth of the nucellus and integuments. By 17 days after bloom they have reached 5.71 mm. in size, compared with a maximum of 7.0 for Downer during the season of 1931. From this date until the fruit is nearly ripe there is a slight gain in size, and then a small decrease as the integuments shrink slightly at about the time of fruit ripening.

TULASNE (20) has remarked upon this early attainment of maximum size by the ovules of *Prunus spinosa* L., *P. avium*, and *P. mahaleb*, the ovule of the first reaching a length of 5 mm. before fertilization. PÉCHOUTRE, too, mentions that the ovule of *P. cerasus* L. reaches almost full size at a very early stage of fruit development.

3. MEGAGAMETOPHYTE AND EMBRYO SAC.—The stages in megaspore formation and megagametophyte development have been studied by RUEHLE (16) for the sweet cherry, by BRADBURY (2) for the sour cherry (*P. cerasus*), for several species of *Prunus* by PÉCHOUTRE (15), and by others. The development as carefully pictured and described by BRADBURY for the sour cherry seems identical with the development of the sweet cherry, so that a critical comparison would be superfluous. Accordingly only the time relationship will be emphasized.

The megaspore mother cell is first distinguished 14 days before full bloom, at the time the individual flower buds have separated in the cluster. It lies beneath five to eight rows of parietal cells, made up of the "coiffe épidermique" and the "calotte" of PÉCHOUTRE. It may remain in this stage for several days before reduction division. Following this division, disintegration of the three distal megaspores in the linear tetrad occurs rapidly. Thus the stages most commonly observed are either that of the megaspore mother cell or of the functional megaspore.

The megagametophyte is fully developed during the following 7 days, or 3 to 7 days before full bloom, at about the time the petals of the blossoms are showing. Development to the 7-celled stage is rapid. The polar nuclei, however, do not migrate to the center of the megagametophyte until about the time of full bloom, and then fuse a day or two prior to fertilization. A few starch grains are found in the megagametophyte at this time.

The synergids persist for some time, even 5 to 10 days after fer-

tilization. On the other hand, the antipodals disintegrate almost immediately after they are formed. The megagamete is relatively small and remains close to the micropylar end of the megagametophyte.

In the case of the non-functional ovule, which is commonly arrested in development just before full bloom, the megagametophyte may persist unelongated but otherwise complete for 10 to 14 days longer. Even after the nucellar tissue has collapsed, the integuments have shriveled, and degeneration has begun in these parts, the nuclei of the megagametophyte and of the megagamete may remain apparently unchanged, although arrested in growth.

At the time of fertilization there is a rapid elongation of the megagametophyte inclosed by the megaspore membrane. During the season of 1931 this increase in length was from 0.67 mm. 4 days after full bloom to 4.73 mm. 13 days later. It passes easily through the central core of elongated cells (fig. 1), as though in response to turgor pressure within, to assume a characteristically long tubular shape noted as long ago as 1675 by MALPIGHI (11), and called by him "vas umbilicale" and "umbilicus." Such acceleration is even more rapid than the growth of either the ovary or of the nucellus and integuments. It continues for a period of 17 days after full bloom, at which time the embryo sac, inclosed by the megaspore membrane, extends nearly to the chalaza. Development may begin 24 hours before pollen tubes can be found within the ovary, and therefore before fertilization has been accomplished; or it may be delayed until 4 or 5 days after full bloom, at which time a very small embryo can be distinguished.

This rapid development of the megagametophyte and embryo sac has been described by TULASNE for several species of *Prunus*, including *P. avium*, by PÉCHOUTRE who called it a "canal," and by WENT.

Occasionally the chalazal end swells to assume a balloon-like shape, and usually there is more or less narrowing about midway, similar to that described by WENT as an isthmus. WENT also pictures the antipodals as persistent at the chalazal end of the elongated embryo sac. This situation has not been observed in these studies. There is, however, a characteristically large endosperm nucleus

present at the tip of the elongating embryo sac at the chalazal end, which gives the embryo sac a pointed appearance.

Representation of the embryo sac by some investigators as a much-branched haustorial organ ramifying the nucellar tissue near the chalazal end, or as a much-enlarged balloon-shaped lacuna, is probably due to swelling and to artifacts occasionally produced in fixation. Such conditions are not found in fresh material.

The megaspore membrane, which is the outer layer of the embryo sac, is continuous and persistent. With dissecting needles it can be removed intact from fresh material, and still later in development the embryo, cellular endosperm, and embryo sac containing free-nuclear endosperm may all be removed together as though surrounded by the same continuous membrane. TULASNE (20) has also remarked upon the persistence of this membrane in dissection. The last vestiges of it may still be seen pressed against the chalazal portion of the nucellus by the developing endosperm 36 days after bloom.

The haustorial nature of the embryo sac will be discussed in connection with endosperm development.

4. FERTILIZATION.—Fertilization is as pictured for the sour cherry by BRADBURY (2). The sweet cherry, however, is self-sterile. In the case of emasculated flowers, hand-pollinated with tested pollen of known compatible varieties, pollen tubes had penetrated the style only slightly 24 hours after pollination. They were traced to the base of the style 48 hours after pollination in selfed flowers, but no further. In the case of cross-pollinated flowers, pollen tubes were found within the ovary, and occasionally within the micropyle at this time. In all open-pollinated blossoms, numerous pollen tubes could be found traversing the style, penetrating the ovary, and within the micropyle. The effect of emasculation and bagging is to retard the development of all parts from 4 to 6 days but otherwise to produce no unfavorable results.

5. ENDOSPERM.—Both the embryo and the endosperm develop very slowly for a considerable time after fertilization. The primary endosperm nucleus divides prior to the division of the zygote as described by BRADBURY for the sour cherry, and by PÉCHOUTRE.

It remains free-nuclear for some time (fig. 2). The nuclei of the chalazal region are large and relatively far apart. This portion never becomes multicellular. The nuclei of the micropylar region are smaller, close together, and this portion subsequently becomes cellular.

In *Pyrus*, the embryogeny of which is similar to that of *Prunus*, it has been shown by OSTERWALDER (14) that one of the first two endosperm nuclei migrates toward the micropylar end of the embryo sac and is the nucleus from which the cellular endosperm is subsequently derived; while from the other is formed the large-nuclear endosperm of the chalazal region. That this is probably the situation in *Prunus* is suggested by the material.

WENT has described the isthmus of constriction which divides the embryo sac midway into regions. PÉCHOUTRE discusses the elongate embryo sac containing free-nuclear endosperm from the standpoint of rapid movement of food materials from the chalazal region of the nucellus to the embryo and cellular endosperm in the micropylar end of the embryo sac. RUEHLE considers the embryo sac as a haustorial organ, and traces the movement of food materials from the vascular bundles of the integuments through the cells of the chalazal region and through the elongate embryo sac. OSTERWALDER considers the embryo sac of *Pyrus* as serving a haustorial purpose. He suggests that the difference in the shape and size of the endosperm nuclei at opposite ends of the embryo sac is due to nutritive conditions. He also suggests that cell walls are not laid down beyond the isthmus toward the chalazal region because of the chemical make-up and nutritive function of that region.

Cell walls begin to appear the 9th day after full bloom between the nuclei surrounding the embryo at the micropylar end of the embryo sac. Cell wall formation subsequently continues slowly until the 17th day after full bloom, the cellular endosperm reaching a length of only 0.57 mm. at this time during the season of 1931. The cell walls are formed progressively from the micropylar to the chalazal end, and from the periphery inward, much like the deposition of layers of material upon the inside of a pear-shaped bag until it becomes a solid mass (fig. 3). At the region of the isthmus reported by WENT cell walls are sometimes formed, and then often with sev-

eral nuclei in one cell. In the region of the chalaza no cell walls are formed. The increase of the endosperm after it has formed this cellular mass is still from the micropylar toward the chalazal end, but is by cell division rather than by the addition of cell walls to free-nuclear endosperm.

This period of delayed development is followed by a period of very rapid enlargement in which the cellular endosperm increases from 0.57 to 4.8 mm. between the 17th and 31st day after full bloom. There is an active region of cell division at the periphery of the cellular mass of endosperm (fig. 5) away from the micropylar end. The newly formed cells enlarge and are in turn digested by the advancing embryo. There is no indication of the endosperm being "pushed ahead" of the developing embryo, as has been stated in the literature. In the thinner portions of the endosperm it may be only 6-8 layers of cells thick. At maturity, the seed is entirely surrounded by a thin layer of endosperm 0.15 mm. thick at the chalazal end.

6. EMBRYO.—The first division of the zygote is to form a large suspensor cell. The suspensor is small, as described by PÉCHOUTRE and RUEHLE, and similar to that described for the sour cherry by BRADBURY. It is persistent to maturity of the embryo. The development of the embryo is even slower than that of the endosperm. Four days after full bloom in 1931 the embryo had passed the zygote stage, but from then until the 24th day after full bloom it had reached a total length of only 0.16 mm. In the early stages of embryo development, the embryo sac contains many tiny starch grains as reported by BRADBURY.

At this time there occurs a sudden rapid development so that the embryo increases in length from 0.71 mm. on the 27th day after full bloom up to 3.00 mm. on the 31st day. Material killed and fixed during this period shows numerous mitotic figures of various stages throughout the embryo. Development from this point is regular but less rapid. The end result is an embryo with two well developed cotyledons, surrounded by a thin layer of endosperm and a fine line of nucellar tissue, the whole completely filling the integuments.

DORSEY and McMUNN have noted a similar slow, early development of the embryo in the peach (*Prunus persica*), followed by very rapid enlargement. They give the length of the young embryo in the

Elberta peach as only $1/16$ of an inch two months after full bloom, and then describe a period of rapid development for one month.

7. EMBRYO ABORTION.—When embryo abortion occurs it is observed as a sudden arrest in development of the embryo. The nucel-

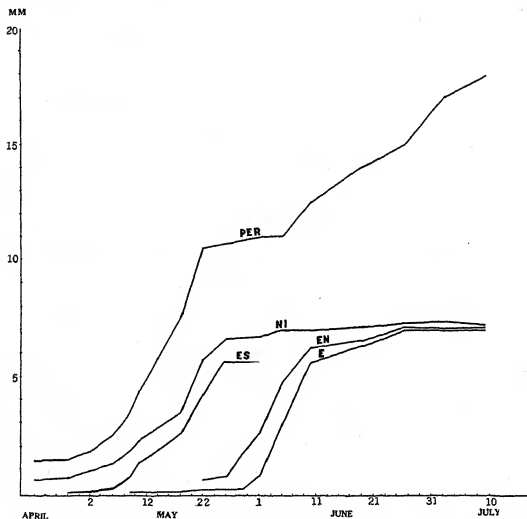


FIG. 9.—Graph of typical development of pericarp (*per*), nucellus and integuments (*ni*), megagametophyte and embryo sac (*es*), endosperm (*en*), and embryo (*e*) of a late-ripening variety of sweet cherry (Downer), which produces a high proportion of viable seed.

lar tissue collapses, the integuments shrivel, and the characteristic aborted seed shown in figures 6 and 7 is the result. The aborted embryo may begin to disintegrate 5 days after arrest in development, or it may remain intact for at least 42 days. There is great variation in the stage of development of the fruit and in the stage of development of the embryo at which abortion occurs.

Critical examination of embryos of dropped fruits from the sweet cherry variety normally producing viable seed has shown no apparent structural variation from a developing embryo of a developing fruit. Neither does there appear to be any structural variation in the

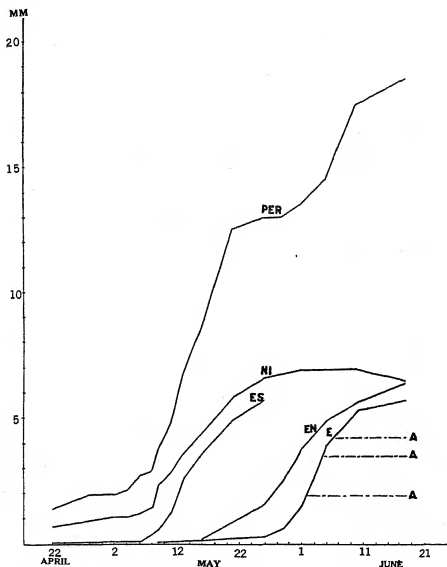


FIG. 10.—Graph of typical development of pericarp, nucellus and integuments, megagametophyte and embryo sac, endosperm, and embryo of an early-ripening variety of sweet cherry (Early Purple Guigne), which rarely produces viable seed.

nucellus or the endosperm of ovules containing abortive seed, with the exception of a suggestion of collapse of nucellar tissue adjacent to the developing cellular endosperm. On the other hand, there is a tendency for the embryo in dropped fruits to continue development,

as reported by BRADBURY. Figure 8 shows an unusual development of an abortive embryo in a dropped fruit. The embryo has "germinated" and has developed six true leaves.

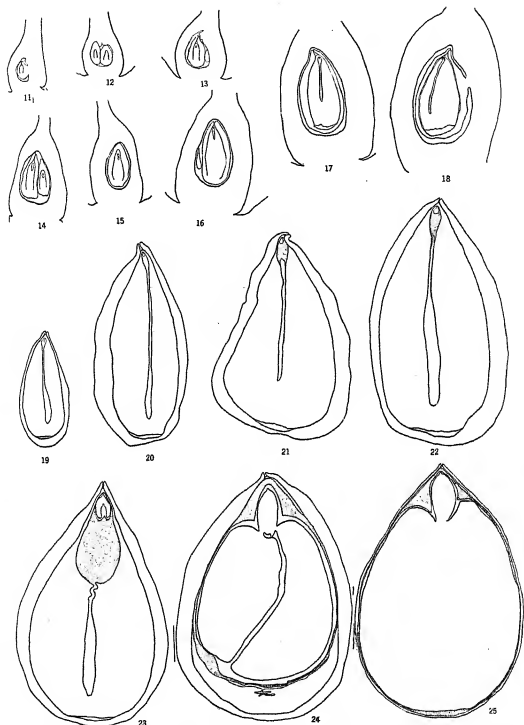
Usually embryo abortion is associated with dropping of the fruit. BRADBURY has shown the various waves of fruit drop for the Montmorency sour cherry and has explained them as resulting from factors of nutrition. KRAUS (8) has pointed out that most fallen fruits contain embryos in some stage of development. DETJEN (5), working with the apple, pear, and plum, has reported that most of the fruits of the June-drop stage contain embryos or tissues resulting from fertilization. He also points out, however, that there is a varietal character involved in the dropping of fruits; that is, trees of the same variety under identical conditions react similarly whereas trees of unlike varieties react differently.

On the other hand, embryo abortion may also be a characteristic of mature fruits of certain varieties. Mention has been made earlier in this paper of the findings in this regard of MARSHALL (12) for the plum; BLAKE (1), CHANDLER (3), CONNORS (4), and SAKAGUCHI (17) for the peach; and WELLINGTON (21) and TUKEY (18) for the cherry.

8. CORRELATION OF DEVELOPMENT.—The development of the various parts discussed in the preceding pages in relation to one another is shown graphically in figure 9. Figures 11 to 25 present camera lucida drawings of development at given stages.

In brief, the ovary and ovule parallel each other in development during the early stages. They develop only slightly from the 55th to the 14th day before full bloom. Between then and the time of full bloom the ovary and the nucellus and integuments begin a more rapid enlargement. The megaspore mother cell is formed at the beginning of this period, and the megagametophyte is in turn formed from it 3-7 days before full bloom. One of the two ovules is arrested in development just before full bloom, and the other takes the ascendancy.

At the time of fertilization there is a sudden acceleration in growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte and embryo sac. Such acceleration continues for a period of 17 days after full bloom. At this time the development of the ovary is abruptly arrested. The nucellus and



FIGS. 11-25.—Camera lucida drawings of stages in development of typical late-ripening variety of sweet cherry (Downer), which produces a high proportion of viable seed. Days before full bloom: fig. 11, 13 days; fig. 12, 7 days; fig. 13, 3 days. At full bloom: fig. 14. Days after full bloom: fig. 15, 1 day; fig. 16, 4 days; fig. 17, 6 days; fig. 18, 8 days; fig. 19, 13 days; fig. 20, 17 days; fig. 21, 18 days; fig. 22, 21 days; fig. 23, 27 days; fig. 24, 36 days; fig. 25, 67 days.

integuments also slow down in growth, but not so sharply. The endosperm and embryo make no appreciable growth.

During the period of retarded development of the pericarp, namely, from the 17th to the 31st day after full bloom, there is a sudden acceleration in growth of the endosperm and embryo. A second period of rapid pericarp development follows the period of retarded development, extending from the 31st day after full bloom to fruit ripening. At this time the nucellus and integuments reach maximum size. The endosperm and embryo continue their development, although at a slower rate. The end result is a ripe fruit containing a viable seed.

Development of an early-ripening variety

For the purpose of tracing the development of an early-ripening sweet cherry, the Early Purple Guigne was selected. This variety is typical of early-ripening sorts which normally produce few or no viable seeds.

1. OVARY.—The ovary increases in length only slightly from the 40th to the 14th day before full bloom. At this time it begins a more rapid period of enlargement, until at the time of full bloom it is 1.94 mm. long.

At the time of fertilization there is a sudden and very rapid acceleration in development of the pericarp which continues for a period of 17 days after full bloom. During the season of 1931 the ovary increased in length from 2.01 mm. the day before full bloom to 2.68 mm. the day following. It measured 3.80 mm. the 5th day after bloom, 4.87 mm. on the 7th, 6.75 mm. on the 9th, 8.5 mm. on the 12th, and 12.5 mm. on the 17th. At the end of this period, development of the pericarp ceases almost as abruptly as it began and a period of greatly lessened growth ensues, which in 1931 extended over a period of 8 days, from the 17th to the 25th day following full bloom. The stony pericarp was becoming brittle at the end of this period, and the fruit was beginning to color.

A second period of rapid pericarp development follows the period of retarded development, commencing on the 25th day after full bloom and continuing until the fruit is ripe, namely, the 37th day after full bloom during the season of 1931.

2. NUCELLUS AND INTEGUMENTS.—Both ovules develop equally but slowly up to 14 days before full bloom, after which there is a more rapid enlargement, and they double in length by full bloom, unless arrested in development.

At the time of fertilization there is a sudden acceleration in growth of the nucellus and integuments. By 17 days after bloom they have reached a length of 5.85 mm. compared with a maximum of 6.96 mm., reached 26 days after full bloom. At various stages, beginning at this time, the nucellar tissue suddenly collapses and the integuments shrivel and collapse. There is no apparent structural abnormality prior to this collapse, with the possible exception of the region of contact between the nucellus and the developing cellular endosperm. At this point there is a loss of turgor in the nucellus just prior to the general collapse of the nucellus and integuments. The phenomenon suggests a sudden decrease in turgor in the thin-walled cells of the nucellar tissue. In this variety seeds are rarely found with integuments which have not collapsed.

3. MEGAGAMETOPHYTE AND EMBRYO SAC.—Development of the megagametophyte and embryo sac is similar in every way to that of the normal variety already described. It progresses regularly and uniformly; no abnormalities being disclosed by a critical study.

4. FERTILIZATION.—Pollination and fertilization are as outlined for the variety already described. Pollen tubes were traced through the style, within the ovary and within the micropyle, in the case of both open-pollinated and also emasculated flowers pollinated by hand. Tested pollen was used of known compatible varieties, as already discussed.

5. ENDOSPERM.—Development of the endosperm following fertilization progresses similarly to that already described. It is free-nuclear until the 9th day after full bloom, at which time the first cell walls appear. Twelve days after full bloom the cellular endosperm has reached a length of only 0.17 mm. By the 22nd day after full bloom, however, a period of rapid acceleration has begun, during which the cellular endosperm reaches a length of 3.73 mm. 28 days after full bloom, and 5.6 mm. 37 days after full bloom.

At various stages from the 28th day after full bloom until fruit ripening, there occurs a sudden arrest in development of endosperm

formation. Structurally there is no apparent change prior to the time of suspended development.

6. EMBRYO.—The embryo also develops similarly to the previous variety. It develops very slowly following the first division of the zygote, until by the 22nd day after full bloom it has reached a length of only 0.25 mm. At this time it begins a sudden and rapid enlargement, so that in some individuals a length of 1.48 mm. has been reached 28 days after full bloom and 4.42 mm. 32 days after full bloom.

Throughout this period of rapid enlargement, however, there is a sudden cessation of growth in various individuals beginning 25 days after bloom, when the embryos are 0.52 mm. in length. Embryos may be arrested in development at any stage (figs. 6, 7). Structurally there is no apparent change in the embryo prior to abortion. The embryo seems merely to be checked in development during its period of most rapid growth. A similar situation has been reported by DORSEY (6) for the plum.

The aborted embryos often develop a peculiar shape, however, as shown in figures 6 and 7. They are often flattened at the tips of the cotyledons, and one tip is frequently curled over the other. It is suggestive of the flattening of the cotyledons against the arrested endosperm and the collapsed nucellus and integuments, as though the embryo had continued its growth for a period after the initial check in development. BRADBURY reported that the embryos of dropped cherries continued their development for some time later. It must be remembered, however, that she was dealing with aborted embryos in dropped fruits, whereas these results deal with aborted embryos in developing fruits.

There is a general grouping of aborted embryos into four waves, not unlike the waves of dropping fruits reported by BRADBURY. One occurs when the embryo has reached a length of approximately 2.9 mm., another when a group of embryos has reached 3.64 mm., again at a stage of 4.20 mm., and again at 5.73 mm. These groups are not absolute, but mark the points of greatest frequency. The writer has found no viable seed from this variety from several thousands that have been examined over a period of several years. The aborted embryos may begin to disintegrate within 5 days, or they may continue in a viable condition for at least 42 days.

7. CORRELATION OF DEVELOPMENT.—Development of the various parts in relation to one another is shown graphically in figure 10. In addition, figures 26 to 40 present development at given stages as camera lucida drawings.

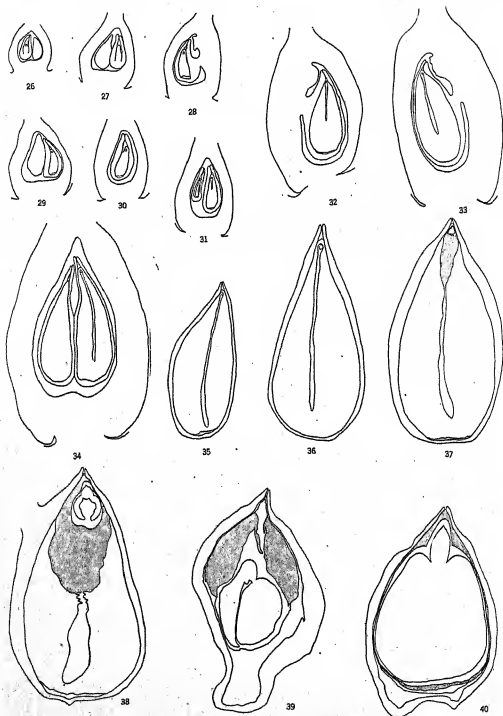
Briefly stated, the ovary and ovule parallel each other in development in the early stages. They increase only slightly in size between the 40th and 14th day prior to full bloom. Between then and the time of full bloom the ovary and nucellus and integuments begin a more rapid enlargement. The megaspore mother cell is formed at the beginning of this period, and the megagametophyte is in turn formed from it 3 to 7 days before full bloom. One of the two ovules is arrested in development just before full bloom, and the other takes the lead.

At the time of fertilization there is a sudden acceleration in growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte or embryo sac. Such acceleration continues for a period of 17 days after full bloom. At this time pericarp development is abruptly arrested. The nucellus and integuments also slow down in growth but not so sharply. The endosperm and embryo make no appreciable growth. During the period of retarded pericarp development, beginning on the 22nd day after full bloom, there is a sudden acceleration in growth of the endosperm and of the embryo.

The second period of rapid pericarp development, which follows the period of retarded development, extends from the 25th day after full bloom until the fruit is ripe on the 37th day. Thus the second period of rapid pericarp development is initiated and the fruit is beginning to color at the time the embryo and endosperm are in their period of most rapid development.

The nucellus and integuments reach maximum size on the 25th day after full bloom. Beginning at this time, and throughout this second period of rapid pericarp development, there is a sudden cessation of both endosperm and embryo development at various stages of development in various individuals.

The pericarp continues its development to the ripening of the fruit. The embryos abort at various stages, and the nucellus and integuments collapse. The end result is a ripe fruit containing a seed with an aborted embryo.



FIGS. 26-40.—Camera lucida drawings of stages in development of typical early-ripening variety of sweet cherry (Early Purple Guigne), which rarely produces viable seed. Days before full bloom: fig. 26, 12 days; fig. 27, 6 days; fig. 28, 2 days. At full bloom: fig. 29. Days after full bloom: fig. 30, 2 days; fig. 31, 4 days; fig. 32, 5 days; fig. 33, 7 days; fig. 34, 9 days; fig. 35, 12 days; fig. 36, 17 days; fig. 37, 22 days; fig. 38, 28 days; fig. 39, 32 days; fig. 40, 37 days.

Comparative development of early and late-ripening varieties

Dividing development into the stages of (1) pre-bloom, (2) pollination and fertilization, (3) first rapid pericarp development, (4) retarded pericarp development, and (5) second rapid pericarp development, the comparison between the early and late-ripening types may be summarized as follows.

During the pre-bloom period, both kinds of varieties develop similarly in all parts. Pollination and fertilization are likewise similar, except that the early-ripening variety develops slightly in advance of and at a slightly greater rate than the late-ripening variety.

During the period of first rapid pericarp development, beginning at the time of fertilization and continuing until 17 days after full bloom, there is also close similarity. During the period of retarded pericarp development the first significant difference between the two types is reached. In the case of the early-ripening variety, this period extends from the 17th to the 25th day after full bloom, or a span of 8 days. In the case of the late-ripening variety the period extends from the 17th until the 31st day, a span of 14 days. The period of rapid embryo and endosperm development begins about the same time for both types, namely, 25 days after bloom.

The period of rapid pericarp development in the early-ripening variety begins at the time rapid embryo and endosperm development are initiated; in the late-ripening variety this period does not begin until 6 days later. That is to say, at the time the second period of rapid pericarp development begins in the early-ripening variety, the cellular endosperm has reached a length of 2.40 mm. and the embryo a length of 0.52 mm. At the time this period begins for the late-ripening variety the cellular endosperm is 4.8 mm. in length and the embryo 3.0 mm.

Furthermore, the increase in pericarp development in the late-ripening variety continues at a much slower rate. It increases 2.6 mm. in 12 days as contrasted with an increase of 4.5 mm. for the first 12 days of this period for the early-ripening variety. Development of the late-ripening variety continues over a longer period of time, until 66 days from full bloom as contrasted with 37 days for the early-ripening variety.

Embryo and endosperm development of the late-ripening variety

is thereafter regular and uniform until a viable seed is produced. During this period embryo and endosperm development of the early-ripening variety is suddenly checked at varying stages of development, and a viable seed is produced only rarely. The nucellus and integuments collapse to give the characteristic shriveled seed of the early-ripening variety as contrasted with the plump, well-filled seed of the late-ripening type.

Results with other varieties

That the situation described for Early Purple Guigne, representing an early-ripening variety, and for Downer, representing a late-

TABLE I

RELATION BETWEEN TIME OF RIPENING OF FRUIT AND VIABILITY
OF SEED OF SWEET CHERRY IN 1927

VARIETY	NO. OF SEEDS	DAYS BETWEEN BLOOMING AND RIPENING	PERCENTAGE VIABLE SEED
Abundance.....	1915	82	94.5
Black Republican.....	3320	80	91.0
Black Tartarian.....	2437	62	7.2
Downer.....	3897	70	98.3
Eagle.....	1850	64	61.9
Early Purple.....	2494	48	00.1
Elton.....	2025	60	00.0
Governor Wood.....	2275	53	7.2
Knight.....	1600	56	00.0
Lambert.....	2220	78	55.0
Mazzard (Geneva).....	4420	86	100.0
Mazzard (Virginia).....	454	Over 80	97.5
Mazzard (Virginia).....	624	Over 80	93.2
Napoleon.....	1809	75	60.1
Oswego.....	3922	95	100.0
Schmidt.....	2396	71	72.8
Windsor.....	1855	79	43.7

ripening kind, is characteristic also of other varieties is shown in table I, taken from a previous publication (18), showing the general relation between time of fruit ripening and viability of seed.

In addition, examination of fresh material during the seasons of 1931 and 1932 concurs with the previous findings. Measurements and critical examination were made under the dissecting microscope at regular intervals during the season. The results are given in table II. They, too, are in general agreement. It will be noted that there

is a tendency for the embryos of the earliest-ripening varieties to abort at an earlier date than those ripening a few days later. Moreover, there is the tendency for the aborted embryos of the earliest-ripening varieties to be smaller than those of varieties which ripen a little later.

TABLE II

RELATION BETWEEN SEED AND FRUIT DEVELOPMENT AND ABORTION
OF EMBRYOS IN SWEET CHERRY IN 1931

VARIETY	NO. OF DAYS BETWEEN FULL BLOOM AND FRUIT RIPENING	LENGTH IN MM. 37 DAYS AFTER BLOOM				NO. OF DAYS FROM FULL BLOOM TO FIRST INDICATION OF EMBRYO ABORTION	PROPOR- TION OF VIABLE SEED
		OVARY	NUCELLUS AND INTEG- UMENTS	CELLU- LAR ENDO- SPERM	EMBRYO		
Burbank.....	48	17	6.8 (collapsed)	4.0	3.1	31	None
Governor Wood...	48	16	6.0 (collapsed)	5.2	4.0	34	None
Lyons.....	49	18	7.2	5.2	4.8	38	None
Knight.....	52	16	6.7 (collapsed)	6.2	5.9	35	None
Elton.....	56	19	7.9	6.0	5.3	37	None
Black Tartarian...	55	15	7.3	5.8	5.2	43	Some
Black Eagle.....	60	14	7.2	5.8	5.2	50	Most
Schmidt.....	64	15	8.0	6.2	5.3	No abortion	Most
Black Republican.	68	15	7.9	6.2	5.9	" "	Most
Mazzard.....	68	9	7.0	5.5	5.3	" "	All
Abundance.....	75	11	6.8	5.0	4.8	" "	All
Oswego.....	77	11	6.9	4.8	" "	All

Discussion

The sudden acceleration in growth of the ovary, the nucellus and integuments, and the megagametophyte or the embryo sac which occurs at the time of fertilization is not necessarily a response to fertilization in itself. Such acceleration may begin 24 hours before pollen tubes can be found within the ovary, and therefore, before fertilization has been accomplished, or it may be delayed until 4 or 5 days after full bloom, at which time a very small embryo can be distinguished. On the other hand, this rapid growth may begin similarly with unfertilized ovules and continue for 7 to 10 days. It may also begin temporarily with ovules containing a normal endosperm development but no embryo, and with ovules containing an embryo but no endosperm.

Further evidence on this point is presented by emasculated and unpollinated flowers. They show a similar sudden and rapid growth of ovary, of nucellus and integuments, and of megagametophyte. The megagametophyte elongates, the synergids and megagamete remain at the micropylar end of the megagametophyte, and the fused polar nuclei maintain a position nearly midway the length. Such a condition is shown in figure 4, 7 days after full bloom. No fertilization has occurred, and the style has abscised. However, no blossoms of this type develop to the ripe fruit.

These facts suggest that the initiation of the period of rapid growth is *coincident with* the time of fertilization, but not necessarily *initiated by* it.

CONNORS (4), in discussing embryo abortion in early-ripening varieties of peaches, says: "The probable cause of abortion is the fact that the enlargement of the drupe takes place at the same time as the final period of development of the embryo, due to a heritable character determining the period of ripening. The flesh becomes mature and ripe and abscission takes place before the development of the embryo is completed."

That the last part of this suggestion does not apply to the cherry varieties under discussion in this paper is shown by the fact that embryo abortion may occur 12 days prior to ripening of the fruit, and that it occurs at different dates in different varieties. KRAUS (8) has called attention to the fact that this arrest in the development of an embryo is also found in the case of certain matings of fowls and in some mammals.

Since there appear to be no histological nor developmental changes prior to embryo abortion in the early-ripening type of sweet cherry, except for the initiation of the second period of rapid pericarp development at the time the embryo and endosperm are making their most rapid growth, attention is naturally focused upon this relation; yet it is not possible to say which is cause and which is effect. Whether the pericarp develops more rapidly in the early-ripening varieties because the embryo aborts, or whether the embryo aborts because of the rapid development of the pericarp, these data give no indication.

As a general proposition, however, from the behavior of plants in

general, one would hazard the opinion that if there is any nutritional balance between embryo development and pericarp development, the abortion of the embryo is the factor which determines the rate and time of the second period of rapid pericarp development rather than the reverse. The suggestion is made that abortion of the embryo may not by itself be responsible for the dropping of a fruit. Possibly quite to the contrary, embryo abortion may be a varietal character which is responsible for the earliness with which some varieties ripen their fruit.

Not only is embryo abortion a characteristic of later waves of dropped fruits, therefore, as pointed out by KRAUS, BRADBURY, DETJEN, and others, but it is also a characteristic of some varieties of fruits which normally ripen their fruit on the tree.

From the standpoint of the plant breeder, the establishment of the fact that early-ripening varieties fail to produce seed with fully developed embryos means that in efforts to breed early-ripening sorts, an early-ripening kind should not be used as the female parent. There is the suggestion from the studies of embryo culture by LARBACH (9), TUKEY (19), and others that some progress might be made in this direction. By culturing an embryo from an early-ripening type upon which another early-ripening variety had been used as the male parent, the chances would be increased of securing a new individual with a combination of genes for early-ripening from both male and female parent. Once a horticultural variety is produced, its perpetuation is assured by the usual vegetative methods of propagation, such as budding and grafting.

That various parts of the fruit and seed may be developing at one time and other parts at another is an interesting point. Instead of the ovary, the nucellus and integuments, the megagametophyte, the endosperm, and the embryo all developing at similar times with parallel growth rates, they develop at different periods with varying growth rates. In spite of this seeming complexity and variability, the factors governing development are remarkably constant. That is to say, if the size of any one part is known, it is possible to tell the relative development of any related part at that time. In other words, when the embryo is a given size, the endosperm development bears a constant relation to it, the nucellus, integuments, and the

pericarp as well. This applies not only to any one season but to different seasons in comparison with one another. The graphs of development may vary in steepness and length, depending upon the season, but the relation between developing parts is constant regardless of the season.

The period of retarded pericarp development following a second period of rapid development is a characteristic of drupaceous fruits. LILLELAND (10) suggests that the retarded growth may be ascribed to the competition for food substances used in forming the stone. He considers that the fact that this cyclic type of growth has not been found in pome fruits further substantiates this premise.

Factors underlying embryo abortion as determined from cross-breeding records

Some light is shed on the factors underlying embryo abortion by the plant breeding records of the New York State Agricultural Experiment Station. Over a period of 7 years they show a minimum of crosses involving early-ripening varieties of cherries as female parents. This is because practical plant breeders have been rewarded with no progeny from such crosses, and therefore have purely empirically stricken such crosses from their cross-breeding programs. The few crosses that have been made which involve early-ripening varieties, however, show that when such varieties of cherries are used as female parents, no viable seed is produced, even though the male parent in the cross is a late-ripening kind. On the other hand, when late-ripening varieties of sweet cherry are used as female parents, viable seed is produced. The use of an early-ripening variety as male parent does not alter the results.

Table III gives the results from 13 recorded crosses over a period of 7 years involving early-ripening varieties as either male or female parent. The number of days elapsing between full bloom and ripening of fruit is given in parentheses after each variety. Unfortunately, there are only two reciprocal crosses, namely, between Seneca (35) and Black Tartarian (55), and between Burbank (48) and Lyons (49), and both of these crosses involve early or early mid-season varieties. Yet the figures tend to show that the limiting factor in the production of viable seed is the nature of the female parent and not

the male parent, and that the problem concerns physiology and nutrition rather than genetics.

Correlation between ripening of fruit and abortion of ovule and embryo

It has already been indicated that there is a tendency for the embryos of the earliest-ripening varieties to abort at an earlier date than those ripening a few days later, and that there is a tendency for the aborted embryos of the earliest-ripening varieties to be smaller

TABLE III

PERCENTAGE VIABLE SEED SECURED FROM CROSS-BREEDING IN WHICH BOTH
EARLY AND LATE-RIPENING VARIETIES OF CHERRY ARE USED
AS BOTH MALE AND FEMALE PARENTS

FEMALE PARENT	DAYS TO RIPEN FRUIT	MALE PARENT	DAYS TO RIPEN FRUIT	NO. OF FRUITS	PERCENT- AGE VIABLE SEED
Seneca.....	(35)	× Burbank	(48)	2	0
Seneca.....	(35)	× Lyons	(49)	7	0
Seneca.....	(35)	× Black Tartarian	(55)	13	0
Seneca.....	(35)	× Abundance	(75)	13	0
Early Purple Guigne.	(37)	× Burbank	(48)	10	0
Early Purple Guigne.	(37)	× Lyons	(49)	21	0
Burbank.....	(48)	× Lyons	(49)	20	0
Burbank.....	(48)	× Black Tartarian	(55)	12	0
Lyons.....	(49)	× Burbank	(48)	28	0
Black Tartarian.....	(55)	× Seneca	(35)	456	8
Eagle.....	(60)	× Seneca	(35)	33	32
Eagle.....	(60)	× Republican	(68)	38	26
Giant.....	(65)	× Seneca	(35)	7	57

than those of varieties which ripen a little later. The question arises, therefore, as to any possible relation between fruits of varying degrees of ripeness on any one day on any given tree. That is, is there a correlation between accelerated pericarp development and abortion of embryo within the same variety?

In an attempt to answer this question, samples of fruits were collected each day during the season of 1932. The method was to take all the fruits on a given branch for each sampling. From 10 to 60 fruits were taken in each sample, involving a total of several thousand individuals, from which the data in tables IV and V were secured. The fruits from each variety were divided each day into various classes, according to degree of ripening as indicated by color.

It was at once apparent that there was a greater degree of variability in ripening among the early-ripening varieties than among the late-ripening kinds. The fruits of Early Purple Guigne, for example, within a span of 12 to 18 inches on a given twig, could be divided into green, greenish-yellow, yellow, red tinge, and half red.

TABLE IV

RELATION BETWEEN FRUIT RIPENING IN LATE-RIPENING VARIETY OF SWEET CHERRY AND DEVELOPMENT OF NUCELLUS AND INTEGUMENTS AND EMBRYO

PROGRESS IN RIPENING	LENGTH IN MILLIMETERS									
	JUNE 19	JUNE 20	JUNE 21	JUNE 22	JUNE 23	JUNE 24	JUNE 25	JUNE 26	JUNE 27	JUNE 28
Total pericarp										
Green.....	12.86	13.02	12.92	12.90	13.34
Red tinge.....	13.10	13.10	13.16	13.36	13.52	13.82	14.34
Half red.....	13.82	14.45	14.52
Full red.....	14.64	14.76
Nucellus and integuments										
Green.....	7.76	7.84	7.72	7.84	7.76
Red tinge.....	7.84	7.80	7.76	7.76	7.96	8.00	7.56
Half red.....	8.12	8.21	8.00
Full red.....	7.82	7.76
Embryo										
Green.....	6.52	6.88	6.94	6.80	7.04
Red tinge.....	6.80	6.70	6.90	6.96	6.60	7.12	6.84
Half red.....	7.00	7.30	7.28
Full red.....	7.04	7.12

On the other hand the fruits of Downer, on a given twig, were more uniform in development; that is, nearly all either in the green stage, the greenish-yellow stage, or the yellow stage, and so on. It was only with difficulty that various degrees of ripening could be secured from the late-ripening varieties of cherry, like Downer.

Likewise it was at once apparent from dissections that there was greater variation in size of embryo, endosperm, and nucellus and

integuments within the early-ripening varieties than in the late-ripening ones. The latter were strikingly similar in size and development on any given date.

TABLE V

RELATION BETWEEN FRUIT RIPENING IN EARLY-RIPENING VARIETY OF SWEET CHERRY AND DEVELOPMENT OF NUCELLUS AND INTEGUMENTS AND EMBRYO

PROGRESS IN RIPENING	LENGTH IN MILLIMETERS										
	JUNE 5	JUNE 6	JUNE 7	JUNE 8	JUNE 9	JUNE 10	JUNE 11	JUNE 12	JUNE 13	JUNE 14	JUNE 15
Pericarp											
Green.....	13.58	13.48
Red tinge.....	13.77	13.81	14.40	14.73	14.40
Half red.....	14.70	15.13	15.23	14.64	15.32
Three-fourths red.....	14.22	15.26	15.80	15.10	14.86	15.56	15.88	16.22
Full red.....	14.30	15.56	15.60	16.03	16.41	17.16
Nucellus and integuments											
Green.....	7.15	7.38
Red tinge.....	6.91	7.13	6.80	6.56	7.00
Half red.....	6.42	6.53	6.46	6.40	6.96
Three-fourths red.....	6.36	6.40	6.30	6.73	6.43	6.56	6.88	6.84
Full red.....	5.60	6.60	6.10	6.66	6.46	6.96
Embryo											
Green.....	3.41	3.66
Red tinge.....	3.47	3.74	3.52	3.16	4.65
Half red.....	3.06	2.90	3.90	4.96	4.68	5.54
Three-fourths red.....	2.90	3.63	3.70	3.48	4.73	5.13	5.08	5.44	5.68
Full red.....	1.60	4.58	3.83	5.63	5.76	6.04

As might be expected, the larger fruits on any given date were farthest along in ripening, as shown in tables IV and V and also in figure 41. Yet all flowers bloomed within a span of 2 days, so that it is not possible to lay this difference to lateness in blooming of some flowers as compared with others on the same branch or twig.

Variation in time of pollination or in time of fertilization could be responsible for the variation; but if this is so, it is difficult to understand why the fruits of a variety like Downer developed uniformly, while those of a variety like Early Purple Guigne developed irregularly.

An examination of the nucellus and integuments of the late-ripening variety, Downer, shows this same relation. Larger nucellus and

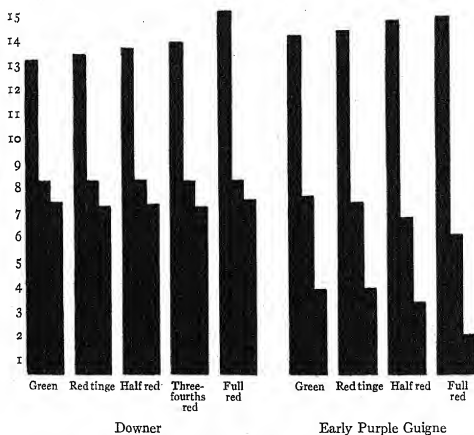


FIG. 41.—Relation between size of pericarp and embryo and nucellus and integuments in fruits of different degrees of maturity picked on the same day. In each column, left-hand section represents the pericarp, center section the nucellus and integuments, and right-hand section the embryo.

integuments are found with larger and riper fruits (table IV). On the other hand, the early-ripening variety, Early Purple Guigne, shows an exactly inverse relation. The largest and most nearly ripe fruits on any given date have the smallest ovules (table V). This fact is most readily seen in figure 41.

Likewise with the embryos, the largest embryos are found in the

largest and ripest fruits of the late-ripening variety, Downer, on any given date, thus correlating large fruit-size and advanced ripening with large size of nucellus and integuments and large size of embryo. And here again, in the case of the early-ripening variety, Early Purple Guigne, the relation is inverse. The smallest embryos are found in the largest and ripest fruits collected on any one day. Yet this inverse relation is not so regular as in the case of the nucellus and integuments. The embryos are often larger, proportionately, than the nucellus and integuments. This suggests that an embryo may continue development for a time independently of development of the nucellus and integuments, as BRADBURY has indicated in abscissed fruits of sour cherry. Figure 8 also shows an embryo which has developed while still contained within the integuments of an abortive seed, in this case producing six true leaves.

The inference to be drawn from these facts is that a check in the development of the nucellus and integuments precedes a check in the development of the embryo. Furthermore, since the embryo seems often to continue development after arrest of other parts of the seed, the underlying cause of embryo abortion is pushed back one step further, namely, to whatever is responsible for cessation of development of the nucellus and integuments. That this is nutritional seems likely, but not necessarily proved by these data.

There is, however, no light shed on whether the check in development of the ovule is due to rapid development of the pericarp, or whether rapid development of the pericarp is in turn due to an abortion of the ovule and the developing embryo. All that can be said is that in varieties of cherries which normally abort the embryo, the largest and ripest fruits on any one given day normally contain the smallest nucellus and integuments and also the smallest embryos.

Artificial culture of normally abortive cherry embryos

Further evidence that the problem is one of nutrition is given by the artificial culture of cherry embryos (19) which was suggested by the study here reported and by the work of LAIBACH and others on the culture of plant embryos. It was reasoned that since there appeared nothing structurally abnormal in aborting embryos, and since embryos in aborted seeds and fruits often continue to de-

velop for a time after abscission, that if given the proper conditions for growth, these aborting embryos might continue to develop. That this has been the case, and that seedlings with true foliar leaves have been grown by this method from normally aborting embryos, show that lack of proper nutritive conditions rather than any hereditary lethal factor has been responsible for failure of early-ripening varieties to produce viable seed.

Summary

1. Both nurserymen and plant breeders experience difficulty in securing seedlings from seed of early-ripening varieties of sweet cherry (*Prunus avium*).

2. Critical developmental studies are reported for a late-ripening type, the variety Downer, and an early-ripening type, the variety Early Purple Guigne.

3. Comparison is made between the development of these two varieties during the seasons of 1930, 1931, and 1932, and also with other varieties: Burbank, Governor Wood, Lyons, Knight, Elton, Black Tartarian, Black Eagle, Schmidt, Napoleon, Black Republican, Abundance, Oswego, and Mazzard.

4. The embryogeny of the sweet cherry is essentially the same as described by BRADBURY for the sour cherry, and as described by PÉCHOUTRE, RUEHLE, and others.

5. The megaspore mother cell is distinguished 10 to 14 days before full bloom, at about the time the flower blossoms have emerged from the bud and have just separated in the cluster. Reduction division occurs several days later. The megagametophyte is formed 3 to 7 days before full bloom, about when the petals are showing.

6. The ovule lengthens but slightly between the 55th day before full bloom and the time of full bloom. The non-functional ovule is arrested in development just prior to full bloom, but the megagametophyte in the aborted ovule may persist unelongated, but otherwise complete, for 10 to 14 days longer.

7. Ovary development parallels that of the nucellus, in which increase in length is slight from the 55th day before full bloom until full bloom.

8. At the time of fertilization there is a sudden acceleration in

growth of the ovary and of the nucellus and integuments, and a marked elongation of the megagametophyte or embryo sac, which continues for a period of 17 days after full bloom. The nucellus and integuments reach nearly maximum size, and the embryo sac reaches nearly to the chalaza.

9. Development of the embryo and endosperm is much delayed. The endosperm remains free-nuclear until the 9th day after full bloom, and is then cellular only for a short distance. Subsequent development is by cell division progressively from the micropylar end toward the chalazal end, and from the periphery toward the center. The free-nuclear endosperm in the chalazal end of the embryo sac does not become cellular.

10. Seventeen days after bloom rapid pericarp development ceases almost as abruptly as it begins and a period of retarded growth follows. The period of retarded pericarp development continues 8 days for the early-ripening variety and 14 days for the late-ripening kind. During this period the embryo and cellular endosperm begin a sudden and rapid development.

11. A second period of rapid pericarp development follows the period of retarded development. It begins 31 days after full bloom for the late-ripening type and 25 days after bloom for the early-ripening type. The embryo of the late-ripening type has developed to a length of 3 mm., whereas the embryo of the early-ripening type is only 0.52 mm. in length.

12. The embryo of the late-ripening variety during this period develops to maximum size, surrounded by a thin layer of endosperm and a line of nucellar tissue, all contained within the two integuments, to give the viable seed characteristic of this type of fruit. On the other hand, in the case of the early-ripening variety, four waves of embryo abortion occur at this time. The nucellar tissue collapses, the integuments shrivel, and the characteristic abortive seeds of early-ripening varieties are the result.

13. Embryo abortion is observed as a sudden arrest in development of the embryo. No apparent structural variation has been observed in abortive embryos, with the exception of the tendency for such embryos to assume a flattened shape at the tips of the cotyledons, with the cotyledons overlapping each other.

14. The first indication of irregularity in development occurs at the region of contact between the nucellar tissue and the developing cellular endosperm. At this point there is a loss of turgor in the nucellar tissue just prior to the general collapse of the nucellus and integuments.

15. Although abortive embryos are frequently associated with dropped fruits, in this case they are associated with normally ripening fruits. Abortive embryos need not necessarily be associated with poor pollination, sterility, incompatibilities, or even nutrition.

16. Whether the pericarp develops more rapidly in the early-ripening varieties because the embryo aborts, or whether the embryo aborts because of the rapid development of the pericarp, these data give no indication. Although all parts do not develop at the same rates and during the same periods, yet they bear the same relation to one another even in different years.

17. The sudden acceleration of parts at the time of fertilization is coincident with the time of fertilization but not necessarily initiated by it. Emasculated and unpollinated flowers show the same sudden rapid development of ovary and of nucellus and integuments, and the marked elongation of the megagametophyte as in the case of hand-pollinated and fertilized blossoms, but do not continue to the formation of ripe fruit.

18. The effect of emasculation and bagging is to retard the development of all flower parts by from 4 to 6 days.

19. In the central zone of the nucellus, reaching from the chalaza to the base of the megaspore mother cell and the megagametophyte, a core of elongated cells is formed which suggests a conductive system, and which is the tissue through which the megagametophyte and embryo sac elongate in their subsequent development.

20. Early-ripening varieties of sweet cherry should not be used as female parents in the breeding of new varieties. Breeding records involving late-ripening and early-ripening varieties of cherry show no progeny when early-ripening varieties are used as female parents, but do show progeny when used as male parents, indicating that the problem of production of non-viable seed is not genetic.

21. Among varieties of cherries which normally contain abortive embryos, the earliest-ripening ones contain the smallest embryos.

22. The largest and ripest fruits on a given branch of a late-ripening variety on any one day contain the largest ovules and embryos. This relation is exactly the reverse for early-ripening varieties, in which the ovules and embryos are smallest in the earliest-ripening fruits. These facts indicate that a check in the development of the nucellus and integuments precedes a check in the development of the embryo.

23. Further evidence that the problem is one of nutrition is given by successful artificial culture of normally abortive embryos.

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ORIGIN AND DEVELOPMENT OF TISSUES IN EQUISETUM SCIRPOIDES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 440

MARION A. JOHNSON

(WITH PLATE VIII AND TWENTY-NINE FIGURES)

Introduction

Equisetum scirpoides is the smallest representative of the genus *Equisetum*. It is a circumpolar species extending in North America from Labrador to Pennsylvania, westward to Illinois and thence northwestward into Alaska. The general appearance of the plant is that of a mat or dense tuft, formed from a tangle of rhizomes which give rise to aerial shoots bearing branches from their lower nodes. The stems are seldom straight, being twisted and intertwined, thus contributing further to the matlike habit.

The internal stem structure is similar to that characteristic of the genus, in that there is a formation of supranodal wood from which the bundles below alternate with those above, a loss of protoxylem with the resulting carinal canals, and a cortex with chlorophyll-bearing tissue and prominent vallecular cavities. The bundles and vallecular cavities are typically three in number, although in the rhizome four not infrequently occur. The pith is not disrupted as in the larger species but remains intact.

Equisetum has long attracted the attention of botanists on account of its structure and phylogenetic relationship with Calamites and Sphenophyllales. Despite this interest, no complete account of the origin and development of tissues for *E. scirpoides* has apparently yet appeared. It is the purpose of this paper to present such an account.

Since a relatively small portion of the work on *Equisetum* is concerned with *E. scirpoides*, only brief mention of the literature will be made. The older accounts up to 1864 are ably summarized by DUVAL-JOUE (17), while the more recent papers have been referred to by BROWNE (7). Since then BROWNE (8-11) has made further

anatomical studies on the cone and stem. In 1920 BARRATT (4) presented additional material on the vascular system. The gametophytes of several species have been studied by WALKER (36, 37) and by ASAHINA (1). SCHAFFNER (24-26) dealt with the problem of evolution in *Equisetum* and with the geographic distribution of the species in relation to their phylogeny.

Materials and methods

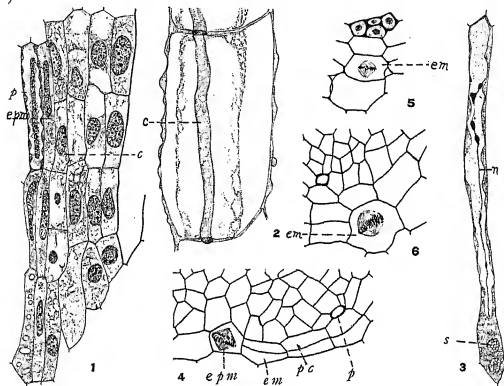
Collections of whole plants were made by Miss MILDRED FAUST of Syracuse University from an Arbor Vitae swamp near Syracuse, N.Y. Additional material was kindly supplied by the director of the Brooklyn Botanic Garden. Vegetative tips and roots were obtained in abundance from plants growing in soil and from others placed in water. A formalin-acetic-alcohol mixture was found to be a satisfactory killing agent; plasmolysis was slight and mitotic figures, especially of the metaphase, were numerous. Siliceous materials were removed by treatment with hydrofluoric acid. Both the usual histologic technique of dehydration and imbedding in paraffin and the butyl alcohol method as proposed by ZIRKLE (39) were used. The latter gave somewhat better results, but in either case cutting was facilitated by soaking in water for several days. Safranin and light green, and safranin and fast green, were found to make excellent staining combinations. Either light green or fast green proved to be valuable in differentiating the phloem before the other vascular elements could be recognized with certainty.

Observations

APICAL CELL AND ITS DERIVATIVES

The axis of the aerial shoot is terminated by the free surface of a pyramidal apical cell which by anticlinal walls cuts off segments from its three lateral faces. Transverse sections taken immediately below the apical cell show three nearly equal segments extending to the center. As in the ferns, each of these is divided unequally by an oblique wall, forming in all six sextants so placed that the larger meet at the center and alternate with the three smaller ones. A few exceptions to this regular arrangement were found in which the major sextants did not alternate but were cut from adjacent sides of the

original segments. Tangential walls soon divide each of the large sextants, forming three inner cells which give rise to the pith. From this point on, both radial and tangential divisions are made in such rapid succession that it is difficult to trace the order of their appearance. Median longitudinal sections through the stem tip show, as reported by REISS (23), that each segment from the apical cell



FIGS. 1-6.—Fig. 1, portion of longitudinal section of stem showing limit of stele and cortex: *p*, phloem; *epm*, endodermis-pericycle mother cell; *c*, cortical cells; $\times 500$. Fig. 2, longitudinal section through endodermis at node: *c*, Casparian strip with vacuoles; $\times 2000$. Fig. 3, sieve tube showing: *s*, lateral sieve plates; *n*, disintegrating nucleus; $\times 1280$. Fig. 4, transverse section of stem showing endodermis-pericycle mother cell giving rise to pericycle and endodermis mother cell: *pc*, pericycle; *em*, endodermis mother cell; $\times 500$. Figs. 5, 6, portions of transverse sections of stem showing endodermis mother cell dividing to form endodermis and layer of cortex; $\times 500$.

undergoes anticlinal and then periclinal division, forming two tiers of two cells each, the upper ultimately developing into the node and the lower into the internode. The inner cell of each tier gives rise to pith while the outer ultimately produces cortex and stele, although it is impossible to determine whether the separation of these tissues is accomplished by the first periclinal division. At first the

derivatives are alike in appearance, but at the third or fourth node from the tip two distinct regions can be seen (fig. 1). The cells in the outermost region are approximately one-half as long and twice as broad as those in the innermost one, possess spherical nuclei, and have large vacuoles and a fibril-like cytoplasm. This doubtless is the cortex. The innermost region on the other hand is made up of long narrow cells with elongated nuclei and dense granular cytoplasm free from large vacuoles. Subsequent differentiation proves this to be the stelar tissue.

PITH

The pith, which is cut off from one of the original segments of the apical cell by the first periclinal wall, divides at once by both periclinal and anticlinal walls. This early activity extends through the second or third nodes, below which general maturity is evident. The cells are not disrupted as in the larger species, but have their walls thickened with a green staining material and supplied with numerous simple pits.

PHLOEM

As a rule the protophloem is the first of the vascular elements to be recognized. It is differentiated from a prominent procambial strand which makes its appearance in the node, and then extends down into the internode and up into the leaf, where near the tip it is reduced to several cells closely resembling the remaining leaf parenchyma. The order of differentiation is the same as that for the procambial strand, and when complete forms a continuous connection throughout the leaf, node, and internode. At maturity the protophloem caps the vascular strand as one or two layers of small, thick-walled cells which have somewhat the appearance of fibers. Careful examination of the lateral walls reveals the presence of sieve plates with very small pores (fig. 3).

Stages in the development of the protophloem show a gradual elongation of the cell, accompanied by the deposition of thickenings on the walls and a decrease in the density of the cytoplasm. The nucleus keeps pace with the elongating walls until it is hardly more than a ribbon of nuclear granules surrounded by a membrane. The elongation of the nuclei is striking, lengths of 230μ being not uncom-

mon. In some cases the nucleus loses its membrane, takes on a slimy appearance, and displays a few spindle-shaped bodies which stain red with safranin (fig. 3). In still other cells the nucleus disappears entirely, which seems to be the normal condition at maturity.

Metaphloem if present is not distinguishable from the phloem parenchyma, which in turn cannot be differentiated from the xylem parenchyma.

XYLEM

The protoxylem first becomes lignified in the upper part of the internode, but soon develops throughout the internode and ultimately into the node, making connection with the leaf trace below. A second center of lignification occurs high in the leaf sheath, extending both up into the leaf and down into the sheath until union is made with the protoxylem of the stem. The order of differentiation in the leaf trace seems to differ somewhat from that described by BARRATT (4), who found that lignification in *E. arvense* began at the tip. The individual protoxylem elements consist of tracheids with spiral or annular thickenings, except in the node, where transitions from spiral to spiral-reticulate and even reticulate markings occur, as has been noted by VIDAL (35) in *E. palustre*. Elongation in the internode is so rapid that the protoxylem is early disrupted with the formation of the characteristic carinal canals.

In vigorously growing shoots the metaxylem is differentiated in the sixth internode from the tip. It consists of from one to seven cells flanking the protoxylem, and is continuous throughout the node, internode, and leaf trace. The greatest display, however, is found at and above the node, where it forms a band of supranodal wood which may be two to four cells in thickness (fig. 31). The tracheid walls are conspicuously marked with scalariform thickenings and multiseriate pits.

The order of lignification is endarch in the stem and mesarch in the leaf, as reported by EAMES (18).

ENDODERMIS AND PERICYCLE

The endodermis is composed of a single layer of cells completely inclosing the stele and continuing through both the node and internode. It also surrounds the leaf traces, extending up into the leaves

until it comes in contact with the epidermis, which in some instances is so complete that a Casparian strip is common to both (fig. 12 d). The pericycle is generally one layer in thickness, although occasionally it may be doubled. This, however, was found to be a very rare occurrence. No particular activity in forming new tissues was found, except that not infrequently a pericycle cell matures as metaxylem adjacent to the endodermis.

The young pericycle and endodermis cells are of the same length; later those of the latter begin to divide by cross walls, so that at maturity its cells are often three or more times as long as those of the pericycle. Stages in the development of the Casparian strip were not observed. It was found, however, to be composed of a delicate band of homogeneous material with one or more rows of tiny vacuoles (fig. 2).

The problem of determining the origin of the endodermis is complicated by the alternation of the vascular bundles at the node. By using the protophloem as a point of reference, and knowing that at maturity the pericycle and endodermis are each one layer in thickness, it is possible to locate the endodermis and pericycle long before the Casparian strips are developed. Cross-sections show the young pericycle cells paired with the stelar elements, and in turn the endodermis paired with the cortex as well as with the pericycle. This situation suggests that the endodermis might be derived from either stele or cortex, or perhaps from both. Definite mitotic figures were discovered which show a single cell (fig. 4 *epm*) giving rise to both pericycle and endodermis mother cell. The endodermis mother cell (figs. 5, 6 *em*) in turn forms the endodermis proper, together with a layer of cortex which may also divide. The question arises as to whether the pericycle-endodermis mother cell belongs to stele or cortex. An examination of longitudinal sections through a bundle two or three nodes from the apex reveals a single cell (fig. 1 *epm*) just outside the protophloem which, on account of its length, elongated nucleus, and finely vacuolated cytoplasm, is regarded as belonging to the stele. This cell corresponds with *epm* of figure 4, which upon division gives rise to pericycle and endodermis mother cell; thus it follows that the pericycle, endodermis, and at least one layer which is regarded as cortex is stelar in origin.

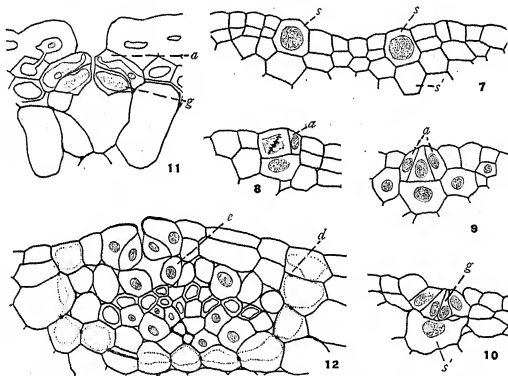
EPIDERMIS AND CORTEX

The epidermis is made up of long, somewhat irregular, close-fitting cells with exceedingly thick walls. Siliceous materials are deposited in and on the walls, especially the outer tangential wall which has an undulating appearance due to uneven thickening. A single uniform hypodermal layer occurs in the furrows of the stem, but is doubled on the ridges, where the cells are larger and the walls thicker.

In the furrows of the stem and leaf sheath the epidermis is interrupted by two prominent rows of stomata, whose origin can readily be traced in transverse sections taken immediately above the nodes of actively growing stems. The earliest recognizable stage consists of a cell which is much larger than its neighbors. A tangential division results in the formation of two daughter cells (fig. 7 *s*, *s'*). The outer cell (*s*), by two slightly oblique radial walls, as shown in figures 8 and 9, gives rise to the accessory cells (*a*) flanking the true stoma mother cell. This mother cell is divided equally by a radial wall to form the guard cells (fig. 10 *g*). The substomal cell (*s'*) keeps pace with the enlarging stomal unit until organization is complete; then a cleavage is noted in the wall common to it, the accessory cells and the guard cells (fig. 10). Separation of the walls continues until the outer face of the substomal cell has been freed. The wall between the guard cells splits also, forming the stoma. During subsequent growth the substomal chamber is enlarged and the epidermis overarches the stomal unit so that it lies in a shallow pit (fig. 11). This encroachment by the epidermal cells displaces the accessory as well as the guard cells, and they become oriented with the long axis tangent to the circumference of the stem. At maturity the accessory cells are covered with siliceous tubercles and the guard cells have the well-known bands found in the genus.

On the upper surface of the leaf, directly over the midrib, there are two or three rows of stomata which differ from those found elsewhere (fig. 12). The accessory and guard cells are only slightly cutinized, and the cells adjacent to the substomal cavity are free from chloroplasts and have the appearance of epithem tissue, as seen in hydathodes. The hydathodal nature is further suggested by the close association with the vascular bundle, the substomal chamber open-

ing upon the xylem, and the presence of an endodermis which makes contact with the epidermis on either side of the stomata-bearing region, thus surrounding the whole unit and separating it from the neighboring chlorophyllose cells. That these stomata are hydathodes can easily be demonstrated by placing plants in a saturated atmosphere. Under such a condition drops of water appear on the upper surface of the leaves. Since there are no other natural open-



FIGS. 7-12.—Figs. 7-11, transverse sections through stems showing stomata in various stages of development: *s*, mother cell of stomatal unit; *s'*, substomal cell; *a*, accessory cells; *g*, guard cell; $\times 375$. Fig. 12, transverse section of leaf showing water stoma: *e*, epithem tissue; *d*, Casparian strip common to wall of endodermis and epidermis; $\times 375$.

ings above the leaf sheath, it is evident that the stomata in question have a secretory rôle. If the endodermis serves here as a waterproof layer, this is a striking example of an arrangement for the exudation of water without flooding the intercellular spaces of the surrounding mesophyll.

The cortex proper is composed of four or five layers of radially arranged chlorophyll-bearing cells. Stages in maturity first occur in the upper part of the internode, and extend downward until a more

or less permanent meristematic region is reached at the base. This basal meristem contributes to both stele and cortex. Three prominent vallicular cavities, whose formation is accomplished by separation and rupture of the parenchyma cells under each furrow, traverse the internode.

LEAF AND LEAF TRACE

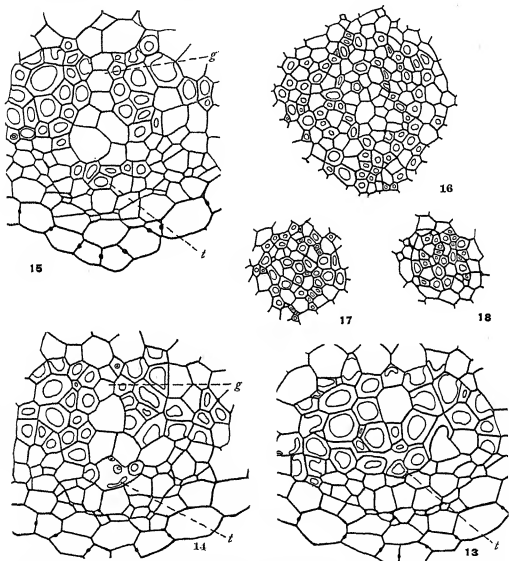
The origin of the leaves can be traced to apical cells which are equidistant from each other on a crown of meristematic tissue a few segments below the apical cell of the shoot. This crown of tissue arises from division and growth in the region of the node, and develops into the sheath bearing the three reduced leaves. Each leaf contains a single vascular bundle, with the exception of the first whorl of the lateral branches which lacks a vascular supply. The chlorophyllose tissue is poorly developed even though the leaves are persistent.

The leaf traces take their origin from the node, where each of the bundles begins to diverge. A careful study of transverse sections in serial order reveals, in some cases, a gap in the supranodal wood immediately above the trace (figs. 13-15). In other instances the gap may be closed by a single metaxylem cell, or there may be no evidence whatever of a break in the xylem. Communication between pith and cortex is never made because of the presence of a well-developed endodermis. It has been definitely determined that these gaps are not due to lack of maturity; some of the most striking ones were seen in old stems which were fully developed and were even bearing branches from the upper nodes.

BRANCH

The branches, as previously noted for *Equisetum*, arise in the axil of the leaf sheath between the leaves instead of in the leaf axil as in other plants. The mother cell of the branch, which is in the shape of a rectangular prism, is oriented with its long axis at right angles to the leaf sheath and is the outermost cell from a series of periclinal divisions, being thus truly exogenous. Later its shape is modified by the growth of the leaf sheath, so that it is broader at the base than at the exposed tip. The first division occurs parallel to the longitudinal axis, forming two cells of about the same volume. The upper one

gives rise to a row of isodiametric cells, while the lower one becomes the branch initial proper. By further growth the initial assumes the shape of the original mother cell, with the lower part of the outer face



FIGS. 13-18.—Figs. 13-15, series of transverse sections through mature stem showing departure of leaf trace and reduced condition of metaxylem: *t*, leaf trace; *g*, gap in metaxylem; $\times 475$. Fig. 13, bundle before departure of leaf trace. Fig. 14, leaf trace still attached to bundle; note gap in metaxylem. Fig. 15, leaf trace free from bundle; note persistent gap. Fig. 16, transverse section through cone axis immediately below first whorl of sporangiophores showing arrangement of xylem; $\times 350$. Figs. 17, 18, same as fig. 16, except from above first and second whorl of sporangiophores respectively; $\times 350$.

somewhat rounded, owing to the pressure exerted by the developing leaf sheath. The first division of the initial itself is perpendicular

to its longitudinal axis, cutting off a basal cell which divides repeatedly so that its progeny cannot be distinguished from the cells lying just beneath. Continued division in this region forces the initial upward, forming a much sharper angle (fig. 19) with the main stem than is found in the other species of *Equisetum*. A series of three divisions now cuts up the initial into as many segments, forming at the same time the pyramidal apical cell which divides in the same manner as does the apical cell of the main axis (fig. 20).

The base of the leaf sheath grows up around the branch, covering it completely, which makes it necessary for the branch to force its way through several layers of tissue before appearing outside (fig. 21). It was not determined whether mechanical force or digestive activity or a combination of both is employed in breaking through the sheath.

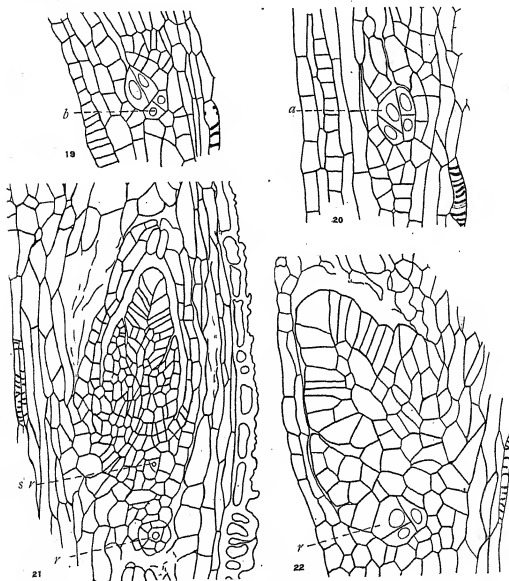
The occurrence of dormant branches is not at all uncommon; in fact they can be found at practically every node. In the lower ones they are well developed (fig. 30); in the upper ones, however, growth appears to be arrested but organization is complete. Their viability may be demonstrated by cutting stems into lengths of one node each and placing in water. In this way one or more branches may be induced from each node.

No evidence of a branch gap could be found. The departure of the trace leaves the supranodal wood undisturbed. In figure 31 the unbroken cylinder of xylem at the point of departure of the trace can clearly be discerned.

RHIZOME

The rhizome, while similar in general structure to the aerial shoot, exhibits a somewhat greater diameter, usually four vascular bundles, longer internodes, and four well-developed leaves in each whorl instead of three. The origin and development of the various tissues seems to be the same as in the aerial stems. One noteworthy feature is the reduction of metaxylem in the internode so that each flank consists of from one to four cells. It is not uncommon to find bundles in which the metaxylem of one flank is entirely absent for several millimeters. In such cases the other flank remains normal (fig. 28). No instance was found in which a flank was absent throughout the

length of an internode. The reduction of the metaxylem in the internode has been noted previously. QUÉVA (22) states that it may sometimes be completely absent from the internodes of rhizomes of *E.*



FIGS. 19-22.—Fig. 19, longitudinal section through young branch; initial has cut off two segments (one in plane of the paper); note basal cells (*b*); $\times 340$. Fig. 20, same as figure 19 except that initial has cut off three segments and formed a typical pyramidal apical cell (*a*); $\times 340$. Fig. 21, longitudinal section of completely formed branch: *r*, first root; *sr*, later developed root; $\times 180$. Fig. 22, *E. arvense*: longitudinal section of branch showing origin of root: *r*, root initial; $\times 340$.

maximum and *E. limosum*. BROWNE (10) is of the opinion that the reduction in metaxylem is correlated with the relatively small number

of branches that are developed. While this may be true, it must be remembered that in *Equisetum* each node is supplied with roots, which doubtless reduces the movement of water through the internodes to a minimum. Furthermore, the roots are not attached to the rhizome proper but to buds, so that the aerial shoots have their own root supply almost from the beginning, and are therefore practically independent of the rhizome. It would seem, therefore, that reduction of the metaxylem is not only correlated with the small number of branches but also with the independence of the node and aerial shoots. In either event the transpiration stream between internodes would be small, which might in itself bring about reduction of the metaxylem. Certainly the reduction of xylem under such conditions would be no hardship in the plant. It is interesting to note that reduction is confined to the xylem, while the phloem remains unchanged.

The occurrence of tylose-like growths in the carinal canals is a unique feature of the rhizome and one which has been reported but twice previously for the genus (SYKES 32, STRASBURGER 30). The parenchyma cells bordering the canals proliferate to such an extent that they are completely filled; in cross-section they appear as patches of irregular cells with large nuclei and cytoplasm heavily laden with starch grains (fig. 32).

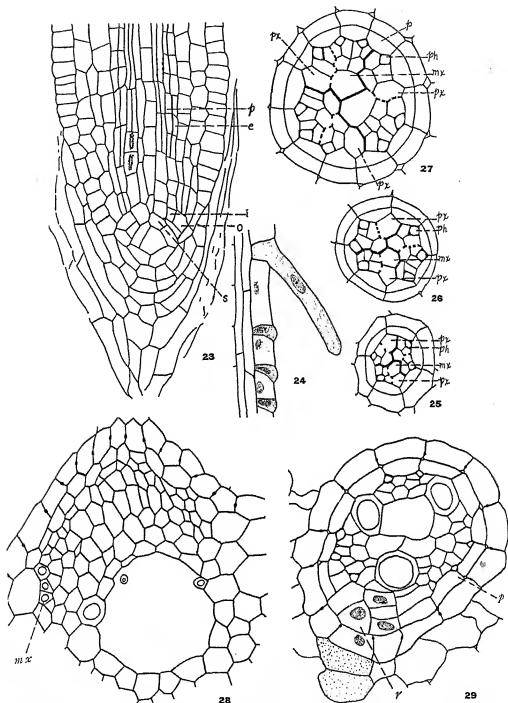
Root

The roots are about 0.5 mm. in diameter and often reach a length of 20 cm. or more. They are brown in color, and are covered for several centimeters back from the tip with a dense growth of root hairs which remains intact long after the tissues have reached full maturity. A prominent root cap protects the apical region.

The roots in the mature sporophyte arise only in connection with the lateral branches, this being true for both the aerial stem and the rhizome. The root initial may be traced to the lowest segment in the first series of three cut from the apical cell. This segment divides by anticlinal and periclinal walls, as described for the stem tip, so that two tiers of three cells each result. The upper tier develops into node and leaf sheath, while the two outer cells of the lower add to the base of the leaf sheath and the innermost cell enlarges and becomes the root initial. The initial is isodiametric in shape, has a large nu-

cleus and dense cytoplasm; and by three intersecting walls gives rise to a pyramidal apical cell whose three lateral faces contribute to the root proper, while the outer one forms the root cap. This method of root formation was found to be true of *E. arvense* also (fig. 22). The first root in each of these species is clearly endogenous; other roots may arise at the base of the leaf sheath as shown in figure 21. Their origin was not determined, however, although it also is definitely endogenous as to position.

The tissues of the root, as has been stated, are derived from an apical cell with four cutting faces. The outer segment is early divided by radial walls into four cells, from which the massive root cap is developed by subsequent divisions. Each of the three internal segments divides by a periclinal wall, separating stele and cortex, and a second periclinal wall soon follows, cutting off the epidermis (fig. 23). The epidermal cells divide at right angles to the long axis of the root, and ultimately become much elongated, with the exception of the root hair initials. These may be early recognized by their small size, large nuclei, dense cytoplasm, and their failure to elongate with the other epidermal cells. Back of the region of elongation they become papillate and grow out into characteristic root hairs (fig. 24). The remaining cortical cell after separation of the epidermis divides periclinally. The outer cell (fig. 23 *o*) develops into typical cortical tissue some four or more cells in thickness; two or three of the external layers ultimately have much thickened walls (fig. 35). Prominent intercellular spaces occur adjacent to the endodermis, so that the stele appears to be suspended by the inner layer of cortical cells (fig. 35). The inner cell (*i*) gives rise to pericycle and endodermis (figs. 23, 33). At maturity the endodermis is marked with the characteristic Casparian strips; while the pericycle, despite its cortical origin, is identical with that of the stem except that its cells remain paired with those of the endodermis. Lateral roots, as in other plants, have their origin in the pericycle, a single cell of which increases in size and divides three times, forming a pyramidal root initial with its base adjacent to the endodermis (fig. 29 *r*). Both the endodermis and cortex opposite the initial are stimulated to divide. Growth in these tissues, however, is not rapid enough to accommodate the young root, and it soon breaks through the cortex.



FIGS. 23-29.—Fig. 23, longitudinal section of root showing origin of tissues: *i*, inner cortical cell from original segment; *o*, outer cortical cell from same; *e*, endodermis; *p*, pericycle; *s*, first stelar cell from original segment; $\times 275$. Fig. 24, longitudinal section through epidermis of root showing root hair and three root hair initials; $\times 180$. Figs. 25-27, diagrammatic representation of central cylinder of root showing tissues contributed by each of the six sextants: *e*, endodermis; *p*, pericycle; *ph*, phloem; *mx*, metaxylem; *px*, protoxylem; $\times 375$. Fig. 28, transverse section from internode of rhizome showing bundle with one metaxylem flank (*mx*); $\times 300$. Fig. 29, transverse section through root showing origin of rootlet from pericycle: *p*, pericycle; *r*, rootlet initial; $\times 300$.

The stele is developed from the inner cells (fig. 23 s), cut from the three original segments, and can best be followed in transverse section. Each cell is divided by a slightly oblique radial wall, forming two unequal daughter cells, the larger of which extends to the center. This is the familiar sextant stage described for other species of *Equisetum* and the pteridophytes in general. Almost at once each of the large sextants divides by a tangential wall, the three inner cells thus formed generally remaining undivided, even in the oldest roots (figs. 27, 34, 35). From this point on two distinct types of stelar development can be recognized. The first type, shown semidiagrammatically in figure 25, is found in the smaller roots. Here one of the small sextants fails to divide and matures as a protoxylem cell; each of the other two contributes a protophloem tube and two or three parenchyma cells. Each of the two large sextants forms a central cell, which remains as parenchyma, a protophloem tube, and two parenchyma cells. The remaining large sextant divides once, forming an inner lignified metaxylem cell and a protoxylem cell. The protophloem tubes are nearly always accompanied by two parenchyma cells, which might be considered as phloem parenchyma except that occasionally one of them adjacent to a protoxylem cell becomes differentiated into protoxylem. This type of root is reported by CHAUVEAUD (14) in *E. ramosissimum*. In somewhat larger roots an increase in the number of protophloem tubes frequently occurs (fig. 26). The small sextant contributing protoxylem and the large one giving rise to both protoxylem and metaxylem remain unchanged. The second small sextant contributes an additional phloem unit, and the third contributes two internal parenchyma cells. Likewise the third large sextant forms an additional protophloem tube and one parenchyma cell.

The second type of development was found in the largest roots (fig. 27), each of the small sextants differentiating to form a protoxylem cell, a phloem unit, and usually an internal parenchyma cell; while each of the large sextants contributes a metaxylem cell, two phloem units, and from one to several internal parenchyma cells. This striking equality in contribution from similar sextants seems not to have been noted in other species of the genus.

CONE

The reduced size of *E. scirpoides* is seen in the cone as well as in the other organs. The largest cones are rarely over 10 mm. in length or 2 mm. in diameter. The fertile sporophores consist of three or four whorls of three members each; a final sterile whorl is imperfectly fused into a hard protective cap. Basipetal protection is furnished the young cone by three large persistent leaves from the stem. The collar or annulus is present, but is much reduced and devoid of vascular tissue. When the spores have reached maturity, the internode between the protective leaves and the first whorl of sporangiophores elongates, thrusting the cone forward and slightly to one side, thus facilitating spore dispersal.

The vascular bundles at the base of the cone, as would be expected, are identical with those of the stem. As the first whorl of sporangiophore traces is approached, however, the metaxylem extends tangentially from the protoxylem, forming a flattened band. Shortly after this condition is attained there is a decided tendency for the bundles to join and give rise to a sharply defined cylinder of xylem with occasional parenchyma cells. This wood it must be remembered is below the node and not above, and is found nowhere else in the stem. In some of the largest cones a pair of adjacent bundles may fail to fuse, so that a gap occurs which persists until the traces to the first whorl of sporangiophores are freed.

In large well-developed cones gaps occur above the first, second, and third whorl of sporangiophore traces. There appears to be considerable correlation between the size of the cone axis and the number and size of the gaps, for two gaps are common above the first whorl of traces where the axis is large and only one per internode above the second whorl of traces where the axis is noticeably smaller. The position of the gaps for the most part is directly above the traces; but the relation to the trace is apparent rather than real, and is probably due to a general dying out of xylem. In no case does a departing trace leave a break in the xylem.

The sporophore traces alternate with those in the whorls above and below, as do the bundles in the stem. The bundles above the fourth whorl of traces unite, forming a protostele which dies out in

the tip of the protective cap. The endodermis is absent from the traces and disappears in the final internode.

In the small cones the vascular bundles remain united throughout their whole length into either a cylinder (fig. 16) or a solid strand. A few parenchyma cells may occur here and there, but they are so irregular in occurrence that they cannot be regarded as the gaps so common in cones of *Equisetum*. After the departure of the first whorl of sporangiophores, the stele is so reduced in size that only three or four pith cells remain in the center; and finally the position of the pith is occupied by protoxylem and a few parenchyma cells (fig. 17). A further reduction occurs after the second whorl of traces is given off, there being only about a dozen xylem cells remaining as an irregular but unbroken rod of xylem surrounded by phloem (fig. 18). When the third and last whorl of traces is formed, no further differentiation of stelar tissues occurs.

Discussion

The present observations on the differentiation of the segments from the apical cell agree with those of REESS (23), CRAMER (16), CAMPBELL (12), and VIDAL (35) in that: the first anticlinal wall divides the segments into a lower and upper cell (the node and internode, respectively), and the first periclinal division cuts off pith on the inside and the parent of stele, cortex, and epidermis on the outside.

The derivation of the pericycle, endodermis, and one or more layers of the inner cortex from the stele is similar to that reported by SCHOUTE (27) and BARRATT (3) in *Hippuris*, but is contrary to the classical account of VAN TIEGHEM (33) to the effect that the endodermis in *Equisetum* is cortical in origin. The majority of recent studies indicate a stelar origin for the endodermis; for instance, CHANG (13) has found it thus in *Pteris aquilina*, as has BARCLAY (2) in *Selaginella wildenovii*; but BARTOO (6) found it to be cortical in *Schizaea pusilla*. In general, EAMES and MACDANIELS (19) consider the endodermis to be stelar. In *Equisetum* the situation in the root is entirely reversed, with the endodermis cortical in origin. Furthermore, the work of CONARD (15) on *Dennstaedtia punctilobula*, *Cibotium regale*, *Aspidium molle*, *Lygodium japonicum*, *Onoclea sensibilis*,

Ceratopteris thalictroides, and *Aspidium marginale*; that of BARTOO (5, 6) on *Schizaea rupestris* and *S. pusilla*; and of STRASBURGER (31) on *Lycopodium selago*, seems to be sufficient evidence for regarding the endodermis of the root as cortical throughout the pteridophytes.

The root of *Equisetum* in textbooks and elsewhere is described as having a double endodermis but no pericycle; it is further pointed out that the endodermis is peculiar in that the cells of the inner layer have walls of uniform thickness and therefore lack the characteristic radial thickenings or Casparian strips. STRASBURGER (30) has suggested that these two layers are a phloeoterma in which the outer functions as a true endodermis and the inner as a pericycle. Few indeed have followed this suggestion; largely it would seem because at present the terms endodermis and phloeoterma are almost synonymous, and also because of the importance still attached to HANSTEIN'S theory of histogens. The evident relationship between the inner and outer endodermis is perfectly clear, for both are composed of the same number of paired cells which are definitely derived from the cortex. To those holding HANSTEIN'S theory of histogens, such evidence, together with the fact that there is no other layer which could be designated as pericycle, would be sufficient for regarding the pericycle as absent. The early separation of tissues as implied by the theory of histogens has long since proved unreliable, so that there is no reason why the inner endodermis should not be termed pericycle. On the other hand, as has been pointed out in a previous paper (21), the pericyclic nature of the so-called inner endodermis is shown by its position, continuity with the pericycle of the stem, and its ability to produce lateral rootlets as is the case in the spermatophytes. According to our present accounts *Lycopodium* is the only other pteridophyte which shares with *Equisetum* in the production of lateral roots from the pericycle instead of the endodermis.

It is apparent, then, that the root in *Equisetum* is no longer to be considered unique because of its peculiar double endodermis, but rather because its pericycle is cortical and consists of cells similar in general shape and number to the true endodermis. It will doubtless be necessary to extend the change in terminology herein suggested to the root of *Calamites*, in which the so-called double endodermis of

Equisetum occurs. The inner layer here should in all probability be referred to as pericycle.

While the reduction of xylem is not a new observation for *Equisetum*, it is significant that in the internode of both the aerial stem and the rhizome, the tracheids nearest the protoxylem are the ones which fail to mature. This is further evidence in support of the current view that the bundle has been derived by reduction from a unit structure. The most striking reduction though is in the supranodal wood, which at times may be completely absent above the leaf trace, leaving a definite gap in the xylem. Only the presence of a well-defined endodermis prevents one from regarding this as a true foliar gap. Indeed, such a gap in the wood of an angiosperm with a poorly developed endodermis would be considered as foliar in nature without question. No other species of the genus shows such extreme reduction in the supranodal wood; nor is so near an approach to a foliar gap seen elsewhere in the Lycopsidea.

In discussing the phylogenetic aspects of the supranodal wood in *Equisetum*, BROWNE (10) suggests that its retention is correlated with the vascular supply of the branches. My observations indicate that a marked relationship exists between size of stem, number of buds, and amount of supranodal wood formed. If the stem is large and has but a single branch, the supranodal wood may be entirely absent above the leaf trace; while if there are two buds it will be well developed above the trace between them. But if the stem is small with either one or two buds, a complete cylinder results. BROWNE's view is clearly supported and strengthened by this evidence.

Tyloses in vessels, tracheids, and canals formed by the breakdown of protoxylem elements are of common occurrence in both fossil and living plants. STRASBURGER and SYKES make brief mention of them in the carinal canals of the larger species of *Equisetum*, but even there they are seldom found and no one appears to have noted them in the smaller species. Their rarity is attested by the fact that in my observations on a great number of stems they have been seen but twice—once beautifully developed in all four carinal canals of a rhizome, and again rather sparingly at the base of a cone. It is difficult to understand why they occur so infrequently; conditions for their development would seem to be favorable, for the protoxylem

elements are disrupted before the surrounding parenchyma cells show signs of senility. In this connection it is interesting to note that SEWARD (28) has found thin parenchyma cells in the carinal canals of *Calamites* which may be interpreted as tyloses.

The position and distribution of the stomata is similar to that in the other species. Their origin and development is as described by STRASBURGER (29) for *E. limosum*. The water stomata found on the upper surface of the leaves have not been described before, although WILSON (38) in a popular paper on excretion of water figures a specimen of *E. arvense* illustrating guttation. It would seem that hydathodes might be of considerable significance in plants with a limited vascular system; in this instance it may be that they are a determining factor in the retention of the apparently functionless leaves of *E. arvense* and *E. scirpoides*.

It is generally thought that *E. scirpoides* is an unbranched form. CAMPBELL (12) expresses this opinion, and adds that the existence of rudimentary buds has not been investigated. Careful examination of plants in the field reveals that most of the aerial stems are really branches from the lower nodes of other stems, and that branching is not uncommon even from the upper nodes. The latter is especially true when the stem tip has been injured. One might therefore expect to find dormant or rudimentary buds in normal stems which my work demonstrates in all stages of development, from initials to completely organized branches with nodes, internodes, leaves, and roots. The close association between branch and root has long been noted but has not received due attention. *Equisetum* is unique in that every branch, whether developed or dormant, is a potential plant equipped for immediate growth, as may easily be shown by allowing stems of one or more nodes to stand in water 7-10 days. Roots and an aerial stem soon appear, and these it must be remembered are not regenerated structures but represent growth in organs previously formed, or that were already in the process of development. Under greenhouse conditions, new plants frequently arise from detached stems which have fallen on the moist soil in the bench. It is likely that this method of vegetative reproduction has had and does have significant survival value in *E. scirpoides* and other species growing naturally in moist habitats.

Considerable discussion is found in the literature as to the origin of the roots in *Equisetum*. All agree that they appear in connection with the branches but the position of the root initials is questioned. JANCZEWSKI (20) reported them to be endogenous in *E. arvense* and *E. limosum*; while VAN TIEGHEM (34) later found them to be exogenous in *E. palustre*, and designated an outer cell of one of the original segments as the root initial. VIDAL (35) came to the conclusion that the root initial was exogenous, although he could not identify it nearly so early as VAN TIEGHEM. A comparison of the figures shown by these writers leaves some doubt as to whether a root initial as figured by VAN TIEGHEM could develop as seen in VIDAL's excellent illustrations. VIDAL's figures, however, appear to be the more convincing of the two. He has drawn the root initial on a line of cleavage between branch and main stem, but the branch is so far advanced that it is impossible to determine with certainty whether the initial is arising from a truly exogenous surface or one which obtains that appearance by a cleavage line between branch and main axis. CAMPBELL (12) is of the opinion that the root is endogenous in *E. telmateia*. My observations in *E. arvense* and *E. scirpoides* not only confirm those of JANCZEWSKI and CAMPBELL, but trace the root initial to an inner cell from one of the original segments of the branch. It is clear then that in *E. arvense*, *E. limosum*, *E. scirpoides*, and *E. telmateia* the root is endogenous; while it may well be exogenous as reported in *E. palustre*.

The root tissues are very regular, being almost diagrammatic in their arrangement and development, as has been reported for the other species. The prominently placed central metaxylem cell seen in *E. hyemale*, *E. telmateia*, and others does not occur. Instead the three inner cells from the three large sextants fail to divide, but remain about equal in size although those which mature into metaxylem become slightly enlarged. This shows how the central parenchyma common to the larger roots has been reduced by the failure of these cells to divide.

The anatomy of the cone in *E. scirpoides* differs from that in the larger species in two respects: first, the sporangiophores alternate almost as regularly as the leaves; and second, even though the xylem is much reduced the extensive gaps and meshes due to its dying

out in the large cones are not found. The gaps when present are small and may be entirely absent. This situation is due to the fact that further reduction in xylem would doubtless interfere with the function of the cone, so the amount of xylem remaining is large in proportion to the size of the stele. The structure of the cone shows this to be the case, for as the tip is approached the size of the stele decreases more rapidly than does the number of tracheids. This results in smaller and fewer gaps until they disappear, leaving a siphonostele, while at the extreme tip the xylem is in the form of a solid strand. The formation of gaps, then, is dependent not only upon a reduction of xylem, as has been generally proposed, but upon the failure of the fundamental tissues to reduce in the same proportion. This it would seem is the key to an understanding of the large stellar gaps of the vegetative axis. It is evident that the extent of the meristem and the length of its period of activity before maturation commences determine the size of the stele. In a plant with an apical cell and only primary growth, it is doubtful whether it is possible for the factors of reduction to influence the early activity of the meristem as much as the tissues which mature from it, without inactivating the apical cell and thus stopping all further growth. A stele with extensive gaps is the inevitable result.

Summary

1. The first periclinal division in each segment from the apical cell of the stem separates pith and "primary cortex," which in turn gives rise to stele and cortex.
2. The pericycle, endodermis, and one or two layers of the inner cortex of the stem are stellar in origin.
3. Evidence is presented for regarding the so-called inner endodermis of the root as pericycle.
4. The endodermis and pericycle of the root are of cortical origin.
5. Dormant branches occur as in other species of *Equisetum*. They are exogenous in origin.
6. The first root developed by the branch is endogenous.
7. The stellar parenchyma in the root is much reduced.
8. Hydathodes of the water stomata type occur on the upper surface of the leaves.

9. Rare but well-developed tyloses are found in the carinal canals.
10. Reduction in xylem is extreme, the supranodal wood being frequently absent above the leaf traces, forming a gap in the xylem.
11. The gaps in the xylem of the cone are correlated with the size of the stele and failure of the fundamental tissue to develop.

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EXPLANATION FOR PLATE VIII

FIG. 30.—Longitudinal section through aerial stem showing young branch; $\times 125$.

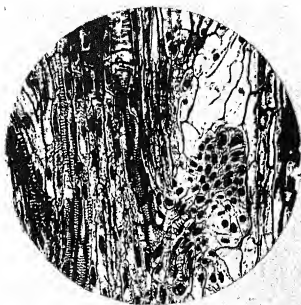
FIG. 31.—Transverse section of mature stem showing vascular supply to one of two branches; $\times 255$.

FIG. 32.—Transverse section through internode of rhizome showing carinal canals filled with tyloses; $\times 190$.

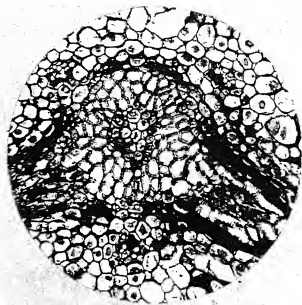
FIG. 33.—Transverse section through root tip showing origin of endodermis and pericycle from same layer of cells; $\times 400$.

FIG. 34.—Transverse section through central cylinder of triarch root; note inner cells from three large sextants remaining undivided; $\times 140$.

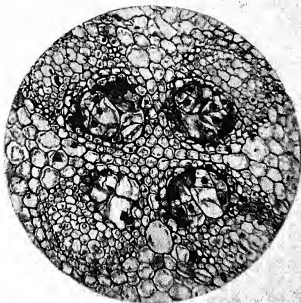
FIG. 35.—Transverse section through diarch root; note stele suspended by cortical cells; $\times 115$.



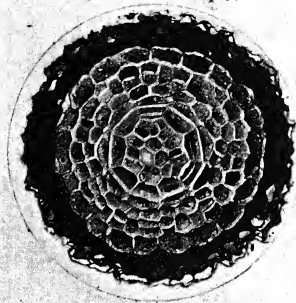
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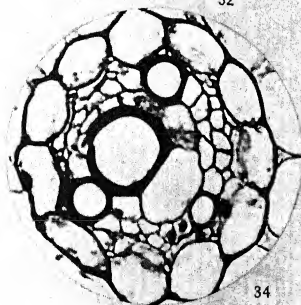
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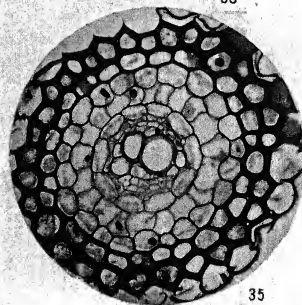
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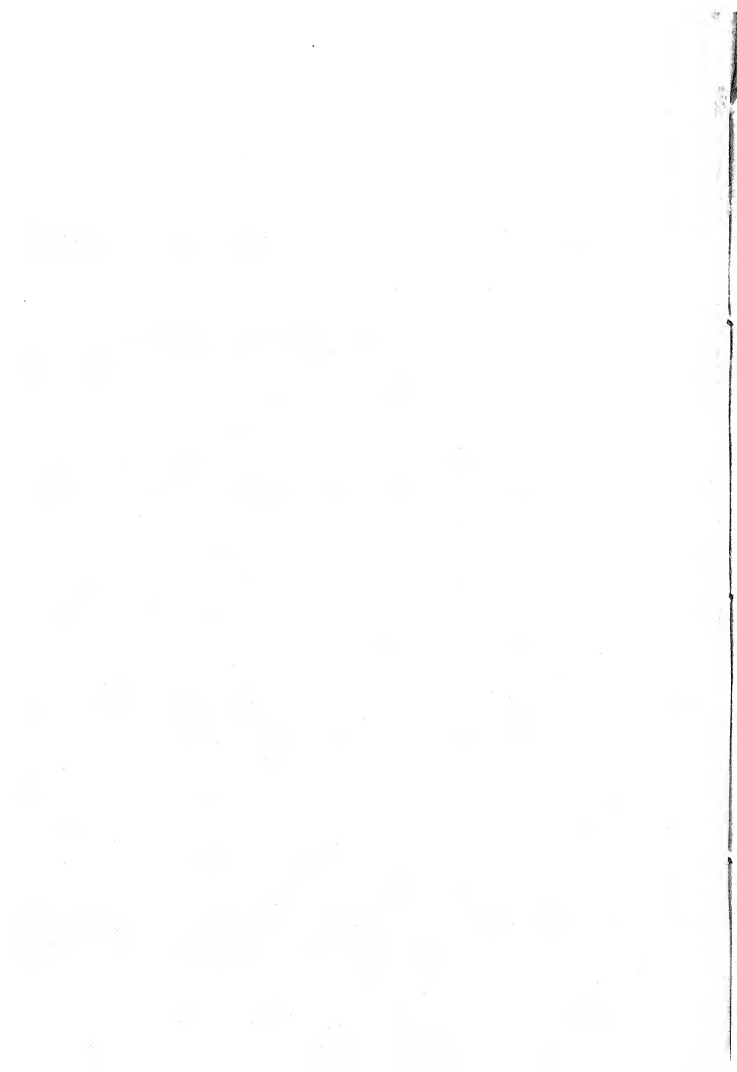


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JOHNSON on EQUISETUM



WALNUT YELLOWS IN RELATION TO ASH COMPOSITION, MANGANESE, IRON, AND OTHER ASH CONSTITUENTS¹

A. R. C. HAAS

(WITH TWO FIGURES)

Introduction

Yellows or little-leaf of walnut trees is a specific chlorotic disease usually considered as being caused by soil conditions rather than having much in common with the diseases known as infectious chloroses. The symptoms of the disease and the nutrition of the trees in health and disease have been discussed by HAAS, BATCHELOR, and THOMAS (7). As with pecan rosette which, according to RAND (14), has much more in agreement with the infectious type of chlorosis, adequate proof has not been given as yet as to the actual cause. Any studies, including the present, merely constitute additional steps in the study of these baffling tree diseases.

These chlorotic diseases differ somewhat in the different hosts. In mottle-leaf of citrus the disease is at its worst in late autumn and in winter, the spring growth frequently being healthy. In pecan rosette the mottling is described by ORTON and RAND (12) and RAND (14) as making its appearance toward midseason, with the later-developed leaves presenting the dwarfed, mottled, and roughened appearance typical of the secondary phase. Toward the end of the season, clusters of dwarfed, depauperate branches are usually put out from dormant and adventitious buds farther back on the branches or main trunk. In walnut yellows, on the contrary, the disease frequently is most severe in the first spring growth, often disappearing entirely in the growth of midseason and late season.

Mottled or rosetted citrus, pecan, and walnut trees, when planted in good soil, are known to recover in most cases; conversely, healthy trees, when planted in diseased soil, most frequently show symptoms

¹ Paper no. 276, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

of disease. HAAS, BATCHELOR, and THOMAS (7) found healthy trees of the three species showing their own peculiar type of chlorosis when planted in a guard row of badly mottled citrus that received sodium nitrate, dried blood, and steamed bone-meal as fertilizer. In the field, all or only part of a walnut tree may show symptoms of yellows, and these symptoms may disappear or become more or less severe in succeeding years. In citrus or walnut nursery rows, trees may be affected with disease while adjacent trees but a foot or more away may be healthy, even though the root systems are almost interlocked. Furthermore, healthy walnut trees, grown in large tanks containing soil obtained from holes in the field from which large diseased trees were removed, showed no signs of disease under well-drained soil conditions.

The cause of walnut yellows is not understood. As a result of an investigation of gray-speck disease of oats which proved to be a manganese-deficiency disease, SAMUEL and PIPER (15) recently suggested that possibly pecan rosette, walnut yellows, and mottle-leaf of citrus are manganese-deficiency diseases; and the latter investigator (PIPER 13) has since reported on the limited availability of manganese in certain soils. The suggestion that a manganese deficiency may be the cause of these tree diseases is of interest because of their chlorotic nature and the intimate relation of manganese to chlorophyll formation and destruction.

The writer (6) has recently emphasized the importance of a deficiency or an excess of manganese in the nutrition of citrus. It was shown that when citrus trees were grown in sand cultures with a culture solution containing excessive manganese, the leaves had only certain resemblances to mottled leaves. In the absence of manganese, citrus cuttings in solution cultures showed chlorosis regardless of the amount of iron added to the solution at daily intervals, and in no case could the chlorotic leaves be considered typical mottled leaves. These results with citrus, and the fact that nothing is known regarding the manganese and iron content of walnut trees, make it desirable to investigate the occurrence of these and other elements in walnut trees affected with yellows. The present investigation is a continuation of previous nutrition studies in healthy and diseased walnut trees.

Material and methods

The material used in the present studies consisted of walnut bark and leaves (*Juglans regia*). The bark was cut from the trunks about 3 feet from the ground. In many cases an extremely thin outer layer of bark was first discarded before taking the bark samples. In this way it was hoped to avoid external contaminations.

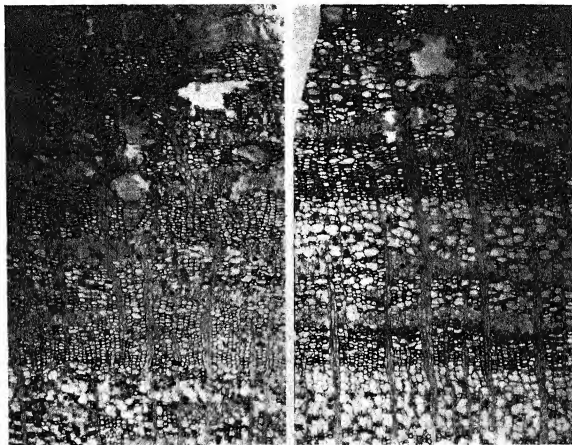


FIG. 1.—Transverse section of walnut tree bark: left, section of healthy tree; right, section of rosetted tree.

The nature of the bark samples used is shown in figure 1. The cells in healthy bark were small and compact, while in diseased bark there was a loose, open structure with many large cells. The bark in both cases contained abundant calcium oxalate crystals.

Care was taken to select only mature leaves for the leaf samples, but even then there was no assurance regarding the average age of the leaves. The results obtained for the leaves, therefore, are not comparable with those obtained by HAAS, BATCHELOR, and THOMAS,

who tagged walnut leaves as they began growth and because of this were assured at least of samples of leaves of similar age.

All samples were dried at 80° C., pulverized in a large porcelain mortar, and dried again at 80° C. Weighed samples were ashed and in most cases determinations were made of the calcium, magnesium, inorganic phosphate, manganese, and iron. Manganese was determined by the periodate method as used by SAMUEL and PIPER, while iron was determined colorimetrically by the method described by ELVEHJEM and HART (4). Calcium was determined by titration with permanganate after double precipitation of the oxalate, while magnesium and inorganic phosphates were determined as pyrophosphates.

Bark

In a preliminary study, walnut trunk bark samples were collected September 27, 1929, from 10-year-old trees at Moreno, California, and on November 2, 1929, from trees of a similar age at Ventura, California. The supply of material obtained was limited. Analysis of the healthy and diseased samples showed the composition as given in table I.

The dry matter of the diseased bark or a water extract contained more ash, calcium, and magnesium than the healthy bark or its water extract. In general the percentages of potassium were greatest in the ash of healthy bark. No definite trend was evident in the percentages of calcium or of sodium in the ash. These results are in agreement with previous studies (7), in which it was found that the composite sap obtained by suction from walnut branches affected with yellows contained more ash and calcium than that obtained from healthy branches.

Another supply of bark material was obtained from 10-year-old trees in a large orchard at Moreno, where a fertilizer experiment conducted by the Department of Orchard Management was in progress. The experimental portion of the orchard consisted of about fifteen rows of twelve trees each. An entire row received a given treatment as regards fertilizer applications. Bark and leaf samples could therefore be obtained from healthy and diseased trees of similar age grown under a given soil management. A thin peel was removed from the samples before they were cut from the trees. The analytical results are shown in table II.

TABLE I
INORGANIC CONSTITUENTS OF HEALTHY AND DISEASED WALNUT BARK (PER CENT)

		VENTURA, CALIF.				MORENO, CALIF.			
		HEALTHY TREE		DISEASED TREE		HEALTHY TREE			
		HEALTHY TREE		DISEASED TREE		DISEASED TREE			

The percentages of ash, calcium, and magnesium in the dry matter, and of magnesium in the ash, were higher in diseased than in healthy bark. The percentages of calcium in the ash did not differ greatly, and later analyses also showed that the slight differences were not consistent. It is evident also that the diseased bark contained more manganese than did healthy bark. These results for manganese do not support the suggestion of SAMUEL and PIPER (15) that walnut trees affected with yellows may be deficient in manganese. In other papers (5, 6) it was shown by the writer that in mottle-leaf of citrus the trees are not suffering from a deficiency of manganese.

In July, 1931, additional bark samples were obtained from other trees in the tree rows at Moreno, and their analytical results are presented in table II.

The percentages of ash, calcium, magnesium, and manganese in the dry matter of diseased bark were again greater than those of healthy bark. The ash of the unpeeled bark showed more iron in diseased than in healthy bark. The results for the peeled samples were inconclusive.

Additional data are given in table III. It will be seen that most of the bark samples obtained on July 20, 1931, were peeled prior to the cutting of the samples. The few additional manganese determinations all pointed to a greater concentration of manganese in the bark of diseased trees. The unpeeled samples showed more iron and phosphate in diseased than in healthy bark. The results for iron and inorganic phosphate in the peeled bark were not consistent. It should be mentioned that in determining iron in bark samples, considerable difficulty was experienced. In most cases the color obtained with thiocyanate changed so rapidly that it was impossible to secure a satisfactory reading, even when phosphate was first removed as was the case in all iron determinations recorded in this paper. Rapid removal of the color with amyl alcohol did not overcome the difficulties. Qualitative analysis showed that the bark samples were rich in copper, and it was found that small additions of copper salts seriously affected the color obtained with thiocyanate and iron. A special study of the relation of copper to walnut yellows and ash composition is contemplated.

TABLE III
MANGANESE, IRON, AND INORGANIC PHOSPHATE CONTENT OF HEALTHY AND DISEASED
WALNUT BARK SAMPLES COLLECTED AT MORENO, CALIFORNIA, JULY 20, 1931

	TREE CONDITION									
	H*	D	H	D	H	D	H	D	H	D
Manganese (p.p.m. in dry matter)	18	20	45	62
Iron (p.p.m. in dry matter)	265	536	918	630	889	312	366	316
Inorganic phosphate (% in dry matter)	792	972	0.21	0.28	0.45	0.42	0.40	0.30	0.24	0.23
	0.28	0.20							0.19	0.19

TABLE III—Continued

	TREE CONDITION									
	H	D	H	D	H	D	H†	D†	H	D
Manganese (p.p.m. in dry matter)	66	104	17	24
Iron (p.p.m. in dry matter)	290	474	637	182	233	294	137	260	1041	1080
Inorganic phosphate (% in dry matter)	0.25	0.27	0.31	0.33	0.20	0.32	0.20	0.33	0.60	0.41

* H, healthy; D, diseased.

† No removal of a thin external layer made prior to cutting bark samples from trunk.

It was mentioned in connection with table III that the unpeeled samples showed more iron and phosphate in diseased than in healthy bark. Accordingly bark samples secured at Moreno on April 20, 1932, were unpeeled except in four samples. It will be seen from table IV that calcium, magnesium, manganese, and inorganic phosphate were generally higher in diseased than in healthy bark. Unpeeled bark usually gave more consistent results than peeled bark. Unpeeled bark samples were thoroughly scrubbed with dry brushes and then wiped clean before the samples were taken, so that contaminations from field sources were at a minimum and no appreciable part of the organic matter of the sample with its ash constituents was lost.

Leaves

Table V gives the results obtained for walnut and a few pecan leaf samples. Even though the samples were collected early in summer and vary somewhat in age, it is clear that manganese was not deficient in either the diseased walnut or pecan leaf samples.

A series of walnut leaf samples were collected at Moreno from the same trees from which the bark samples were obtained, and at the same time. As was pointed out by HAAS, BATCHELOR, and THOMAS (7) for walnut leaves and by HAAS and HALMA (8) for citrus leaves, the inorganic composition is continually undergoing change. The leaves in the present case were not tagged and were random samples of what were considered mature leaves. The ash and calcium in tables VI and VII showed no obvious relation between healthy and diseased leaves. HAAS, BATCHELOR, and THOMAS, with tagged leaves of equal age, found magnesium to be higher in the diseased leaves. The data in tables VI and VII further confirm that diseased leaves contain a higher inorganic phosphate content than healthy leaves. LOEW (10), TRUOG (16), and others have pointed out that magnesium increases the absorption and utilization of phosphorus. Although not emphasized by KELLEY and CUMMINS (9), their data also show an increased magnesium as well as phosphate content in mottled citrus leaves. Magnesium is a constituent of chlorophyll. It would seem that these mottled citrus or walnut leaves, being yellow between the veins, would have less chlorophyll than would healthy leaves and consequently less magnesium. One must not con-

TABLE IV

CALCIUM, MAGNESIUM, INORGANIC PHOSPHATE, AND MANGANESE IN DRY MATTER OF DISEASED AND HEALTHY BARK SAMPLES COLLECTED AT MORENO, CALIFORNIA, APRIL 20, 1932; SAMPLES NOT PEELD EXCEPT WHERE NOTED

	NO SOIL TREATMENT		AMMONIUM SULPHATE 4 LB. PER TREE		COMPLETE FERTILIZER 10 LB. PER TREE		BLOOD 6 LB. PER TREE		MANURE 10 CU. FT. PER TREE		SULPHUR 10 LB. PER TREE		NO SOIL TREATMENT		POTASSIUM CHLORIDE 8 LB. PER TREE				
	H*	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D			
Ca (per cent).....	5.42	5.91	5.45	5.67	4.83	5.75	5.17	5.90	5.79	5.96	5.64	5.85	5.58	5.44	5.90	4.81	5.76	5.28	6.93
Mg (per cent).....	0.1730	0.2800	0.1690	0.2470	0.1850	0.2070	0.2760	0.3380	0.1710	0.2210	0.1880	0.1500	0.2270	0.1740	0.1720	0.1710	0.1680	0.1450	0.103
Mn (p.p.m.).....	48	79	52	58	45	127	58	51	146	119	52	84	138	47	51	132	96	97	108
Inorg. P ₂ O ₅ (p.p.m.).....	509	763	560	718	631	636	602	690	632	600	700	467	675	542	727	569	528	572	473

TABLE IV—Continued

	COPPER SULPHATE 3 LB. PER TREE		PRUNED; AMMONIUM SULPHATE 4 LB. PER TREE		PRUNED; NO SOIL TREATMENT		IRON SULPHATE 3 LB., CALCIUM NITRATE 6 LB., AMMONIUM NITRATE 3 LB. PER TREE						NO SOIL TREATMENT							
	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D		
Ca (per cent).....	5.63	5.57	5.54	6.00	5.37	6.22	5.56	5.98	5.99	5.14	6.14	5.09	6.43	5.70	6.50	5.31	5.42			
Mg (per cent).....	0.1840	0.1740	0.1070	0.2160	0.1800	0.2550	0.1000	0.1300	0.1540	0.1000	0.2320	0.1490	0.2200	0.1620	0.2020	0.1500	0.151			
Mn (p.p.m.).....	62	94	60	146	58	76	64	107	111	92	99	76	132	23	121	39	30			
Inorg. P ₂ O ₅ (p.p.m.).....	671	581	662	669	522	633	548	940	448	563	608	687	720	478	670	684	568			

* H, healthy; D, diseased.

† Extremely thin layer of outer bark peeled off prior to securing samples.

clude that all magnesium in a leaf is associated with chlorophyll, however, for then the concentration of magnesium in a leaf might be in some degree a measure of the chlorophyll contents.

Tables VI and VII show diseased leaves to have a larger manganese and iron content than healthy leaves. A deficiency of manganese in the diseased leaves did not appear to exist as suggested by SAMUEL and PIPER. There is as yet only a very hazy conception of

TABLE V

ASH, MANGANESE, AND CALCIUM CONTENT OF HEALTHY AND DISEASED WALNUT AND PECAN LEAVES, GROWN IN CALIFORNIA AT LOCATIONS INDICATED

	WALNUT LEAVES								PECAN LEAVES						
	JUNE 5, 1925		JUNE 22, 1925			JULY 1, 1925			JUNE 9, 1925		JUNE 22, 1925		JUNE 22, 1925		
	ORANGE COUNTY		ORANGE COUNTY		RIVER-SIDE	WHITTIER			ORANGE COUNTY		ORANGE COUNTY		ORANGE COUNTY		
	H*	D	H	D	D	H	D	D	H	D	H	D	H†	D†	
Ash (% in dry matter)....	8.38	10.42	11.30	7.19	10.34	9.71	10.95	14.60	10.58	7.68	9.65	9.64	8.89	8.58	9.11
Mn (p.p.m. in dry matter)....	92	108	68	50	80	88	44	42	56	144	340	220	240	177	320
Ca (% in dry matter)....	1.50	2.01	2.37	1.64	2.01	1.80	2.62	2.88	2.02	1.86	2.16	2.50	1.90	2.18	2.11
Ca (% in ash)....	17.90	19.33	21.00	22.86	19.43	18.58	23.97	19.70	19.13	24.23	22.39	25.92	21.38	25.38	23.18

* H, healthy; D, diseased.

† Sample consisted of healthy appearing leaflets of basal half of primary petiole.

what availability of an element within the plant actually means. In the case of iron, some investigators find chemical analysis indicating an excess within chlorotic leaves and still the plant may respond to the further application of iron to the soil or its injection into the tree. This has been attributed to faulty distribution within the plant, non-availability, or insolubility. The writer knows of no such situation in the case of manganese.

There is a distinction between the manganese relation in rosetted and in etiolated leaves. BERTRAND and ROSENBLATT (3) found that etiolated leaves of vegetables contained less manganese than green leaves, while tables VI and VII show that rosetted leaves contained a higher manganese content than healthy leaves.

TABLE VI

COMPOSITION OF MATURE LEAVES OF HEALTHY AND DISEASED WALNUT TREES AS DETERMINED ON SAMPLES COLLECTED AT MORENO, CALIFORNIA, SEPTEMBER 25, 1930

	No SOIL TREATMENT		AMMONIUM SULPHATE 4 LB. PER TREE		COMPLETE FERTILIZER 10 LB. PER TREE		BLOOD 6 LB. PER TREE		MANURE 10 CUB. FT. PER TREE		SULPHUR 10 LB. PER TREE		No SOIL TREATMENT		POTASSIUM CHLORIDE 8 LB. PER TREE	
	H*	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Ash (% in dry matter).....	13.11	15.88	13.78	13.86	14.46	11.74	14.09	15.74	13.58	13.43	11.78	12.26	13.43	13.88	13.19	10.26
Ca (% in dry matter).....	3.43	4.31	3.52	3.57	3.62	2.70	3.80	4.07	3.55	3.57	2.86	2.84	3.38	3.42	3.34	4.85
Mg (% in dry matter).....	0.54	0.71	0.61	0.80	0.68	0.61	0.65	0.71	0.48	0.61	0.56	0.59	0.52	0.67	0.57	0.53
Mn (p.p.m. in dry matter).....	183	253	147	517	217	160	207	240	176	220	168	800	180	328	223	260
Fe (p.p.m. in dry matter).....	567	867	800	800	800	400	767	767	633	633	500	767	567	733	717	717
Inorganic PO_4 (% in dry matter).....	0.45	0.76	0.51	0.69	0.39	0.55	0.55	0.88	0.47	0.47	0.46	0.57	0.45	0.62	0.57	0.59

TABLE VI—Continued

	COPPER SULPHATE 3 LB. PER TREE		PRUNED; AMMONIUM SULPHATE 4 LB. PER TREE		PRUNED; NO SOIL TREATMENT		IRON SULPHATE 3 LB. NITRATE 6 LB. AMMONIUM NITRATE 3 LB. PER TREE		No SOIL TREATMENT		No SOIL TREATMENT		No SOIL TREATMENT	
	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Ash (% in dry matter).....	13.84	16.19	13.30	15.10	15.74	15.23	15.36	13.31	14.94	14.66	14.19	17.37	15.66	13.40
Ca (% in dry matter).....	3.74	4.64	3.50	4.06	4.38	4.08	3.88	3.38	3.64	3.36	3.76	5.30	3.84	3.39
Mg (% in dry matter).....	0.57	0.77	0.51	0.70	0.64	0.78	0.62	0.61	0.54	0.59	0.56	0.92	0.64	0.76
Mn (p.p.m. in dry matter).....	227	483	240	310	210	273	154	350	153	207	180	240	213	358
Fe (p.p.m. in dry matter).....	583	717	583	700	700	733	800	800	733	833	666	933	1217	797
Inorganic PO_4 (% in dry matter).....	0.51	0.60	0.49	0.94	0.45	0.77	0.50	1.01	0.48	0.68	0.60	0.57	0.59	0.57

* H, healthy; D, diseased.

TABLE VII
COMPOSITION OF MATURE LEAVES OF HEALTHY AND DISEASED WALNUT TREES AS DETERMINED ON SAMPLES COLLECTED AT
MORENO, CALIFORNIA, SEPTEMBER 25, 1931

	NO SOIL TREATMENT		AMMONIUM SULPHATE 4 LB. PER TREE		CALCIUM NITRATE 6 LB. PER TREE		COMPLETE FERTILIZER 10 LB. PER TREE		BLOOD 6 LB. PER TREE		MANURE 10 CU. FT. PER TREE		SULPHUR 10 LB. PER TREE		NO SOIL TREATMENT	
	H*	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Ash (% in dry matter).....	14.48	14.55	13.34	14.11	15.34	12.95	12.93	13.30	13.41	13.16	11.58	14.46	12.79	15.40	14.43	14.08
Ca (% in dry matter).....	4.13	3.89	3.94	3.96	4.95	3.19	3.93	3.65	4.01	3.59	3.32	4.50	3.62	4.62	4.45	4.11
Mg (% in dry matter).....	0.60	0.63	0.41	0.65	0.57	0.47	0.35	0.57	0.43	0.57	0.32	0.50	0.38	0.51	0.43	0.56
Mg (% in ash).....	4.14	4.29	3.07	4.61	3.72	3.90	2.67	4.28	3.17	4.33	2.76	3.42	2.97	3.31	2.98	3.94
Mn (p.p.m. in dry matter).....																
Fe (p.p.m. in dry matter).....	157	236	147	183	133	60	325	433	140	168	113	164	162	258	200	242
Inorganic P_2O_5 (% in dry matter).....	392	450	233	478	225	367	317	342	291	296	321	417	433	475	225	492
	0.79	0.82	0.70	0.86	0.54	0.88	0.43	0.93	0.42	1.02	0.42	0.59	0.42	0.63	0.49	0.57

TABLE VII—Continued

	POTASSIUM CHLORIDE 8 LB. PER TREE		COPPER SULPHATE 3 LB. PER TREE		PRUNED: AMMONIUM SULPHATE 4 LB. PER TREE		PRUNED, NO SOIL TREATMENT		IRON SULPHATE 3 LB., CALCIUM NITRATE 6 LB., AMMONIUM NITRATE 3 LB. PER TREE		NO SOIL TREATMENT	
	H	D	H	D	H	D	H	D	H	D	H	D
Ash (% in dry matter).....	12.63	12.39	13.51	13.77	13.51	12.91	13.26	12.37	14.73	14.80	12.65	15.15
Ca (% in dry matter).....	3.96	3.66	4.22	4.15	4.24	3.75	4.03	3.80	4.43	4.33	3.81	4.53
Mg (% in dry matter).....	0.54	0.40	0.47	0.58	0.41	0.37	0.68	0.37	0.47	0.45	0.34	0.52
Mg (% in ash).....	4.24	3.23	3.44	4.21	3.00	2.87	5.13	2.95	3.16	3.01	2.69	3.48
Mn (p.p.m. in dry matter).....	117	183	183	400	83	108	100	83	172	112	189
Fe (p.p.m. in dry matter).....	250	217	333	442	383	400	421	358	442	729	917
Inorganic P_2O_5 (% in dry matter).....	0.42	0.56	0.56	0.64	0.44	0.43	0.64	0.41	0.77	0.47	0.79

* H, healthy; D, diseased.

The writer (6), working with citrus cuttings in solution cultures, has found that in most cases less iron was accumulated in the leaves when manganese was made deficient. In rosetted walnut leaves, when manganese was high the iron was also generally higher. McGEORGE (11) found a relation between manganese and iron in

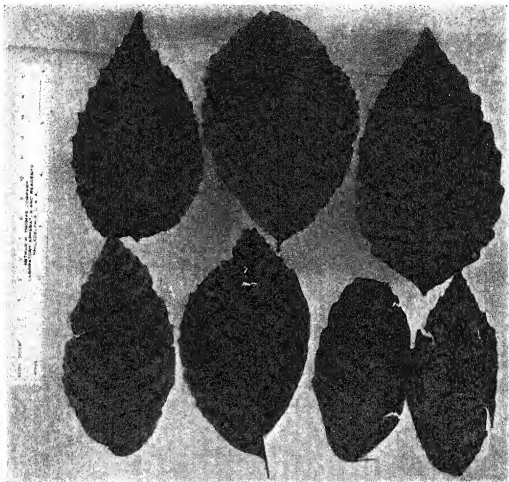


FIG. 2.—Leaflets of walnut seedlings grown in Hoagland's solution containing excessive concentrations of manganese.

sugar cane leaves: less manganese was required if iron was high and less iron if manganese was high.

As a result of dipping or spraying rosetted pecan leaves with ferric sulphate or chloride, ALBEN, COLE, and LEWIS (1) concluded that pecan rosette is a condition of iron chlorosis. ANDERSEN (2) has recently found manganese and copper in chlorotic material of deciduous fruit trees to be consistently less than the amount found

in healthy material. Manganese applications to the soil were without effect but marked response to copper applications was noted.

An effort was made to grow walnut (*Juglans regia*) seedlings in Hoagland's solution to which were added increasing concentrations (10 to 100 p.p.m.) of manganese as sulphate. When any effect was noted, it usually consisted of either a general yellowing of the entire leaf followed by burning along the veins, or a yellowing between the veins followed by eventual burning of the yellow areas. Figure 2 illustrates the effects of excessive manganese. The effects have some resemblance to those of walnut yellows but are far from being typical of the disease (the control leaf is at the left in upper row). Additional experimentation focused about copper and its interrelations with other elements may throw further light on this baffling disease.

Summary

1. A study was made of certain ash constituents in a large number of samples of healthy and diseased walnut leaves and bark. The ash of diseased bark was higher than that of healthy bark. Calcium, magnesium, manganese, and inorganic phosphate were also generally higher in the diseased bark.

2. Walnut leaves affected with yellows contained a higher magnesium, inorganic phosphate, manganese, and iron content than healthy leaves. Diseased leaves, therefore, cannot be considered as containing insufficient manganese unless a considerable amount of that present is unavailable.

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CYTOLOGICAL STUDIES IN THE GENUS HIERACIUM¹

ALTON H. GUSTAFSON

(WITH FIFTY-SIX FIGURES)

Introduction

Hybridism is closely related to such phenomena as polyploidy, apomixis, sterility, certain classes of mutations, and a variety of other conditions of general biological interest. ROSENBERG's (28) investigations of a natural *Drosera* hybrid demonstrated a close correlation between meiotic irregularities and the production of sterile pollen. More extended researches by several workers on a number of genera widely separated taxonomically have shown such a close correlation between meiotic irregularities and known and suspected hybrid forms that the presence of such irregularities in a species has come to be regarded as strong evidence for its origin through hybridization.

Hieracium, one of the largest of genera, displays such an intense polymorphism as causes it to be very difficult taxonomically. Hybrids have been recognized in the genus by many observers. OSTENFELD (23-25), carrying out numerous hybridization and genetical observations, and ROSENBERG (27, 29), investigating the same and similar material cytologically, have demonstrated that hybridization is easily effected in the genus. The cytological work here presented was undertaken in an attempt to add such data as might have a bearing on the important problem of the origin of new forms in nature.

Material and methods

The material, consisting of buds in all stages of development, was collected during the summer months of 1927-1929. Collections were made generally throughout New England and in the provinces of Nova Scotia and Quebec. Herbarium specimens were taken with each collection, in order to check the field determinations. DR.

¹ Contribution from the Laboratories of Plant Morphology, Harvard University.

DAHLSTEDT of Stockholm, Sweden, has checked the identifications in the subgenera *Pilosella* and *Euhieracium*.

The buds were picked on warm sunshiny days between the hours of nine in the morning and three in the afternoon. Carnoy's preserving fluid, as recommended by ROSENBERG (29), was used for killing. An exhaust pump (JEFFREY 11) was used to withdraw the air from the tissue to insure rapid fixation. After 24 hours in Carnoy's, the material was washed in several changes of 95 per cent alcohol and transferred to equal parts of glycerin and alcohol in preparation for imbedding. The material was dehydrated and imbedded in nitrocellulose according to the mass method described by JEFFREY (11). Sections of 5 and 10 μ were made with a THOMSON-JEFFREY sliding microtome. Haidenhain's iron-alum haematoxylin stain was employed, with a weak solution of eosin in 30 per cent alcohol for cytoplasmic contrast.

Preparations were studied and drawn with the aid of a Bausch and Lomb 1.9 mm., 1.30 N.A., oil-immersion objective and a Leitz 20 \times periplan compensating ocular. Drawings were outlined with the aid of a camera lucida from typical stages of pollen mother cells during reduction divisions.

Cytological observations

SUBGENUS STENO THECA

Hieracium venosum L.—ROSENBERG (27) first reported the haploid chromosome number of this species as seven, but later (29) corrected it to nine. The count of nine was confirmed in the present investigation. The chromosomes differ widely in size but the meiotic processes proceed with absolute regularity. At anthesis the pollen grains are all morphologically perfect.

Hieracium paniculatum L. and *H. scabrum* Michx.—The chromosome number, the meiotic processes, and the pollen conditions are as described for *H. venosum*.

SUBGENUS EUHIERACIUM

Hieracium canadense Michx.—This species is a triploid with 27 chromosomes. Pairing at diakinesis is very irregular. The number of bivalents varies from three to 12.

An equatorial plate is rarely formed. Figure 1 shows a side view of the heterotypic spindle at metaphase with seven bivalents and 13 univalents widely scattered. Figure 2 is a similar view with the entire chromosome complement in the form of univalents widely scattered on the spindle. Polar counts at metaphase vary from 12 to 27. Figure 3 illustrates a cell in which 14 chromosomes are visible. Judging from the size of these, seven are bivalent and seven univalent. Figure 4 depicts an unusual condition in which all 27 chromosomes are visible.

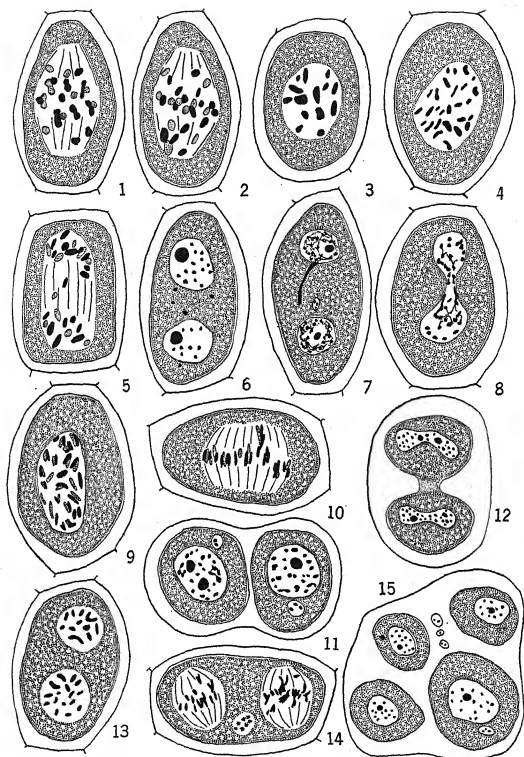
As a result of the irregular metaphase, the distribution of chromosomes to the poles is very unequal. Apparently one member of each bivalent passes to one pole and its partner to the other while the univalents pass to one pole or the other at random. Figure 5 is an anaphase in which 11 chromosomes are found at one pole and 14 at the other, while two are present on the spindle as laggards. As many as 18 have been noted at one pole and as few as seven have been observed. A 13/14 distribution is not uncommon. Lagging is marked. In most cells at least one chromosome and more often several may be seen on the spindle after the majority have reached one pole or the other. The unequal distribution often results in the formation of nuclei considerably different in size. Laggards are frequently extruded into the cytoplasm (fig. 6).

Cells with a number of laggards at anaphase frequently form a "restitution" nucleus which contains the diploid number of chromosomes. Such divisions have been described by ROSENBERG (29) and designated as semiheterotypic divisions. Figure 8 represents the dumbbell-shaped nucleus which often results from a division of this type. Figure 7 illustrates a not uncommon condition intermediate between normal telophase and a semiheterotypic division. The nuclear membranes were able to cut through the spindle, but a long "tail" of chromatin almost connects the nuclei, and a micronucleus has been formed from the laggards. In other cases a chain of several micronuclei was observed lying between the two large daughter nuclei. Now and then two nuclei are formed each with an appendage running toward the other. The membranes have cut through the spindle and the only remaining evidence is seen in the misshapen nuclei.

The "restitution" nucleus of the semiheterotypic division undergoes a further division, as seen in the prophase-like condition of figure 9. A number of chromosomes are split lengthwise in preparation for division. It is probable that all the chromosomes divide in this stage. Figure 10 shows the metaphase of such a division. The spindle is extremely broad and the chromosomes form a nearly regular plate. Figure 11 represents a dyad formed as the result of such a division. Probably some laggards are present, for micronuclei have been formed. Many dyads without micronuclei have been observed. Figure 12 represents a condition found in a number of cells. The shape of the nuclei suggests that the original nucleus had divided although the cells had not become completely separated, following which a semiheterotypic division had taken place in each cell of the dyad.

Nuclei such as those shown in figure 6, or those more or less normally formed as a result of the first division, undergo a second division. Since the number of chromosomes in these nuclei varies considerably, the homoeotypic divisions show corresponding variations in numbers. The greater portion of the nuclei pass through a rather regular homoeotypic division without lagging and with an equal distribution of chromosomes to opposite poles. The result of this second division is a cell containing four nuclei, two of which probably differ in chromosome number from the other two, although all the nuclei may contain different numbers as a result of the conditions mentioned. Polar counts of the homoeotypic spindles often show a distribution of 13 and 14. Polar counts show a variation, however, as the 11 in one spindle of figure 13 and the 16 in the other indicate. Although most homoeotypic divisions are rather regular, a considerable number are decidedly irregular. Figure 14 illustrates an irregular metaphase plate and an extruded mass of chromatin lying between the two spindles. The anaphases may show laggards, and the telophase shows that a certain amount of chromatin is often extruded in this division. This accounts for further variation in the chromosome numbers of the nuclei of the tetrad.

Many of the tetrads appear to be in a state of degeneration. The cytoplasm is thin and highly vacuolated, and the cells are irregular in outline. The nuclei vary in size and present a poorly organ-



FIGS. 1.-15.—Pollen mother cells of *H. canadense* during meiosis. Fig. 1, heterotypic metaphase, bivalents and univalents scattered on spindle; fig. 2, heterotypic metaphase, univalents scattered on spindle; fig. 3, heterotypic metaphase, polar view with 14 chromosomes; fig. 4, heterotypic metaphase, polar view with 27 chromosomes; fig. 5, heterotypic anaphase showing lagging and unequal distribution of chromosomes; fig. 6, interkinesis showing extruded chromosomes; fig. 7, interkinesis showing long "tail" of chromatin and a micronucleus; fig. 8, dumbbell-shaped restitution nucleus; fig. 9, restitution nucleus preparing for division, chromosomes splitting; fig. 10, broad metaphase plate formed as restitution nucleus divides; fig. 11, dyad with micronuclei; fig. 12, dyad with restitution nucleus in each cell; fig. 13, homoecotypic metaphases, polar view showing unequal chromosome numbers; fig. 14, homoecotypic metaphases, irregular plates and extrusions in cytoplasm; fig. 15, polycaric and polysporic tetrad. X970.

ized appearance. It seems that a certain number of potential pollen grains never get beyond the tetrad state.

Polycary and polyspory are frequent features of the later stages of development. Figure 15 illustrates conditions often seen. There are four large cells, no two of equal size, as well as three very small cells. Two of the four large cells contain micronuclei in addition to the normal one, and a third cell shows a small mass of chromatin in the cytoplasm. After the tetrads break up, the young grains often contain one or two small nuclei in addition to the much larger normal one.

These abnormalities indicate that the pollen will present unusual conditions, a high percentage of it being morphologically sterile. Many crushed and empty grains and many microcytes are found in the anthers, together with large grains probably formed from the dyads. It is apparent from the descriptions of the meiotic divisions that the grains may contain chromosome numbers which vary over a considerable range. There are present at anthesis a number of grains which are of normal size, and a considerable number of large grains all apparently sound from the morphological standpoint. No binucleated grains have been found at anthesis, however, although this is the normal condition in species with regular meiotic processes.

The embryos develop apomictically. It is hoped to report upon this more fully at a later date.

Hieracium murorum L.—Herbarium specimens showed the collections falling clearly in the *H. murorum* group but not identical with *H. murorum* of Miss TERRY as reported in GRAY's manual. Dr. DAHLSTEDT pronounced it *H. murorum* (species near *H. praecox*).

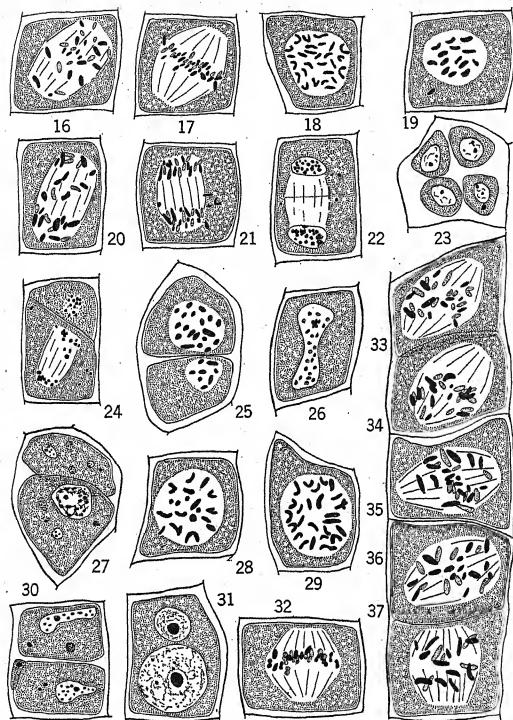
During the prophase there is never a pairing of the chromosomes as is characteristic of the species with regular meiosis. The single chromosomes pass directly from an unpaired condition to the metaphase. Figure 16 represents the usual condition at metaphase, a condition seen in hundreds of cells. The univalent chromosomes are widely scattered on the spindle with no suggestion of the formation of an equatorial plate. Figure 17 illustrates a condition occasionally found. The chromosomes form a regular plate, but more than the expected 27 appear. Apparently some of the univalents have already divided. As might be judged from the preceding figures, counts in

polar view vary greatly. Figure 18 shows a condition observed now and then in which the entire 27 chromosomes are seen. Figure 19 is a similar view in which 14 chromosomes are found in the spindle area and a fifteenth has been extruded into the cytoplasm. Counts ranged from 12 to 27.

The anaphase is also irregular. The foregoing conditions indicate that distribution to the poles is unequal. Figure 20 shows seven chromosomes at one pole and 13 at the other, while seven lie between the poles as laggards. Counts range from seven at one pole and 20 at the other to a 13/14 distribution. Often chromosomes are extruded into the cytoplasm (fig. 21). Interkinesis is illustrated in figure 22. Extruded chromosomes are to be noted in the cytoplasm and a cell plate has been formed; this latter is not formed in species with normal meiosis. Dyads may be formed as a result of the first division, and usually are irregular in some respect. Figure 27 shows one in which a large nucleus has been formed in one cell, together with two small nuclei; and two extruded masses of chromatin are also present. The other and smaller cell contains four micronuclei and a small bit of chromatin. Sometimes no cell plate is formed after the first division, but mostly in such cases the nuclei formed appear to be poorly organized and the cytoplasm is often thin and highly vacuolated.

In many instances a second division follows the first. Figure 24 shows the anaphase of a homoeotypic division in which irregular distribution of the chromosomes, lagging, and extrusion are evident. Figure 25 depicts a polar view of the homoeotypic metaphases. In the lower cell only eight chromosomes are seen, while in the upper 19 are present. The tetrad resulting from the homoeotypic division is shown in figure 23. The cytoplasm is thin and the nuclei are poorly organized. One cell contains a bit of extruded chromatin. Figure 26 illustrates a condition referred to under *H. canadense* as a restitution nucleus. This nucleus rounds up to a normal shape and apparently undergoes division, for several instances were noted in which the chromosomes had apparently divided, as seen in *H. canadense*; but the later stages of division were not seen.

It is curious that in many cells the spindle lies with its axis parallel to the diagonal of the cell rather than parallel to the length, and the



FIGS. 16-27.—Pollen mother cells of *Hieracium murorum* during meiosis. Fig. 16, heterotypic metaphase, univalents scattered over entire spindle; fig. 17, heterotypic metaphase, unusual equatorial plate; fig. 18, heterotypic metaphase in polar view with 27 chromosomes; fig. 19, heterotypic metaphase in polar view with 14 chromosomes on spindle and one extruded in cytoplasm; fig. 20, heterotypic anaphase, irregular distribution of chromosomes and lagging; fig. 21, heterotypic anaphase, extrusion and lagging; fig. 22, interkinesis, cell plate forming, and extrusions; fig. 23, tetrad; fig. 24, homoeotypic anaphases, unequal distribution of chromosomes, and extrusions in cytoplasm; fig. 25, restitution nucleus; fig. 26, homoeotypic metaphases in polar view showing unequal distribution of chromosomes in cells; fig. 27, abnormal dyad.

FIGS. 28-37.—Pollen mother cells of *H. smolandicum* during meiosis. Fig. 28, heterotypic metaphase in polar view with 19 chromosomes; fig. 29, heterotypic metaphase in polar view with 26 chromosomes; fig. 30, dyad with misshapen nucleus, extruded chromatin, small "extra" cell formed; fig. 31, dyad with extreme difference in nuclear size; fig. 32, heterotypic metaphase, equatorial plate formed with dividing univalents; figs. 33-37, cells side by side in same anther; figs. 33-36, heterotypic metaphases with chromosomes widely scattered on spindle; fig. 37, anaphase with portion of cell cut away. $\times 970$.

spindles are often eccentric. Whatever course the divisions may take after interkinesis, the end result is the same in all cases. Dyads, tetrads, or the other varied products of the irregular divisions all degenerate. At anthesis all traces of cell structure have disappeared and the anthers contain only a few bits of entirely degenerate cell fragments. No pollen is formed.

Hieracium smolandicum.—Material was collected along the Big Baddeck River, Nova Scotia, by Professor JEFFREY. Dr. DAHLSTEDT states that it differs from *H. smolandicum* but seems related to that species. A comparison with specimens of *H. smolandicum* subspecies *robinsonii* Zahn in the Gray Herbarium collection makes it rather certain that the specimens are of the same species as discussed here.

The pollen mother cells are similar to those of *H. murorum*, and the same general meiotic irregularities characterize the species. The diploid chromosome number is 27. No pairing of the chromosomes is found at prophase, and the chromosomes appear on the spindle as univalents. Figures 33-37 are especially interesting, for they represent a row of five adjacent cells in the same anther undergoing meiotic division. It is apparent that the chromosomes are all univalents and form no equatorial plate, for they are widely scattered on the spindle. Figure 32 illustrates a condition seen occasionally: The chromosomes form a somewhat regular plate and some of the univalents appear to be dividing. As expected, the polar views vary widely in counts. Figure 28 shows such a view with 19 chromosomes and figure 29 one with 26. Counts ranged from 12 to 26.

As in the case of *H. murorum*, two cells are usually formed after the first division. The dyads produced are generally abnormal (fig. 30). Each cell contains a misshapen nucleus and extruded chromatin. A small bit of cytoplasm containing chromatin has been cut off also. The nuclei are often of very unequal size and micronuclei are usually present. Two nuclei may be formed with no division of the cytoplasm. They are usually unequal in size (fig. 31).

Division figures with all the characteristics of somatic divisions have been observed. The occurrence of a second division has not been observed. No pollen is formed. It is hoped to report upon the apomictic development of the embryos at a later date.

SUBGENUS PILOSELLA

Hieracium flagellare Willd.—Herbarium specimens and collecting bottles were labeled *H. pilosella* var. *viride* at the time of collection. A comparison with specimens at the Gray Herbarium showed similar material had been given the same name, although ZAHN's descriptions indicated that the material was probably *H. flagellare*. Dr. DAHLSTEDT pronounced the material to be *H. flagellare* Willd.

ROSENBERG (29) pays little attention to the pollen formation of *H. flagellare*, but does state that the "pollen formation is typical," with 21 bivalents present. If the term "typical" means normal, the following observations hardly bear out this statement; if it means typical of apomictic species in general, the observations confirm the statement.

The spherical mother cells are surrounded by a heavy callose coat. The great number of chromosomes makes a count at diakinesis of uncertain value. Most of the chromosomes are paired although univalents are also found. Side views of the metaphases reveal irregularities. Figure 38 illustrates such a view, in which the bivalents form a plate which is too compact to allow a certain count to be made. Six univalents are found on various parts of the spindle, and two other constricted chromosomes which appear to be rather large for univalents are found on the spindle also. Univalent laggards vary from one to nine. Usually from 10 to 12 bivalents may be distinguished readily. Figure 39 shows an early metaphase in side view with both univalents and bivalents present. Polar views show counts ranging from 18 to 23. Figure 40 illustrates such a view, in which, judging by size, 14 chromosomes are bivalents and four are univalents. Other counts range from 12 to 15 bivalents and from four to nine univalents.

Heterotypic metaphases are seen in figures 41 and 42. The former shows both bivalent and univalent laggards and extrusion; the latter shows laggards of both kinds, and the univalents appear to be dividing. Figure 43 shows the result of lagging. Several chromosomes have not been included in the daughter nuclei. About 10 per cent of the cells show extrusions in this stage. Occasionally, in the anaphase, a long dark streak is to be noted passing from a split chromosome at one pole to another at the opposite pole, just as though a

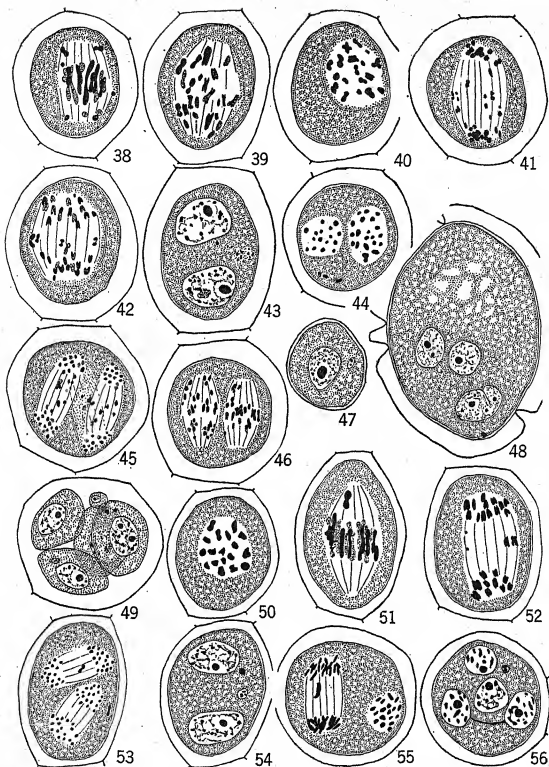
trail of chromatin had been left between the separated members of a bivalent chromosome.

The homoeotypic divisions are also irregular. Figure 46 shows pronounced lagging with chromosomes widely scattered on the spindle. Many cases of normal behavior, or with only one or two chromosomes behaving abnormally, have been observed. The anaphase in figure 45 is very irregular. The center of the cell contains the apparent remains of the heterotypic spindle upon which several extruded chromosomes remain. On each side of this spindle the homoeotypic spindles appear with numerous laggards present. Lagging is more pronounced in the homoeotypic divisions than in the heterotypic. Polar views of the homoeotypic divisions show the variations in numbers which the side views would indicate. Figure 44 shows one spindle with 16 chromosomes while the other has 20, and three large chromosomes are seen extruded in the cytoplasm.

Polycary is commonly noted in the tetrads. The nuclei doubtless contain different numbers of chromosomes, both because of extrusion and because of the presence of both univalent and bivalent chromosomes in the early stages of meiosis. The unequal sizes of the nuclei point to the same conclusion. Polyspory is less frequent. Figure 49 illustrates both polycary and polyspory in the same tetrad.

The pollen grains show great variation in size. A few grains appear empty or degenerate, but the majority, in spite of size differences, appear to be perfect morphologically. Figure 47 shows a young microspore before the tetrad has broken up. The cell is seen to contain an "extra" nucleus. This is a common condition resulting from polycary. Although pollen grains in division show about 20 chromosomes, it has not been possible to establish the chromosome number with accuracy. In a great many cases "giant" grains are formed. Figure 48 illustrates such a grain. In this case three nuclei may be seen, and the lower one is lobed and much larger than the other two. Often the grains are lobulated and decidedly irregular in shape. Cross walls may separate the whole structure into two, three, or four cavities. Judging by the size, shape, and variations in wall structure, these grains have developed from an entire tetrad.

ROSENBERG (29) reports the chromosome number of *H. flagellare* as 42. The exact number was not ascertained with certainty in this



FIGS. 38-49.—Pollen mother cells of *H. flagellare* during meiosis. Fig. 38, heterotypic metaphase, bivalents in plate, univalents scattered on spindle; fig. 39, heterotypic metaphase, univalents and bivalents widely scattered on spindle; fig. 40, heterotypic metaphase, polar view of plate with 18 chromosomes (14 bivalent and 4 univalent); fig. 41, heterotypic anaphase, lagging, extrusion, division of univalents; fig. 42, heterotypic anaphase, lagging, division of univalents; fig. 43, interkinesis with several extrusions; fig. 44, homoeotypic metaphases, polar view with unequal distribution of chromosomes, extrusions; fig. 45, homoeotypic anaphases, chromosomes widely scattered on spindles; fig. 46, homoeotypic anaphases, irregular distribution of chromosomes, lagging, extrusion, remains of heterotypic spindle; fig. 47, microspore with "extra" nucleus; fig. 48, giant pollen grain formed from whole mother cell; fig. 49, tetrad showing extrusion, polycary, and polyspory.

FIGS. 50-56.—Pollen mother cells of *H. pratense* during meiosis. Fig. 50, heterotypic metaphase in polar view with 18 chromosomes; fig. 51, heterotypic metaphase with regular plate of bivalent chromosomes, single univalent laggard; fig. 52, heterotypic anaphase, two bivalent laggards and split univalent laggard; fig. 53, interkinesis with extrusion and micronucleus; fig. 54, homoeotypic anaphases, lagging, extrusion, and unequal distribution of chromosomes; fig. 55, homoeotypic anaphases, spindle at left

study, but there are at least 36 and probably not more than 42 chromosomes.

Aposporic embryo sacs reported by ROSENBERG (29) have been found. Details of their formation will be given later.

Hieracium pratense Tausch.—The pollen mother cells are much as in *H. flagellare*. At diakinesis the chromosomes are distinctly in pairs, although it is not certain that all of them are in this condition. Heterotypic metaphases in polar view usually show 18 chromosomes (fig. 50). Side views of the plate show the chromosomes arranged regularly. About 10 per cent of the cells in this stage show minor irregularities. Figure 51 is regular except for the bivalent laggard at the upper end of the spindle. A single univalent laggard has been seen in some cells. Polar views sometimes show only 17 chromosomes, as would be expected.

Although the anaphases are mostly regular, irregularities do occur (fig. 52) which show several laggards between the groups at the poles. The cell in this illustration has been partly cut away and does not show the full chromosome complement. The chromosomes are clearly split in preparation for the homoeotypic divisions. At the left are two small chromosomes, probably the result of a split univalent. Interkinesis as seen in figure 54 shows the result of lagging. Between the daughter nuclei a micronucleus and a bit of chromatin are seen. Where divisions are normal, of course, as is the rule in this species, the two daughter nuclei are regularly formed.

The homoeotypic divisions are also normal for the most part. Figure 53 shows a case with minor irregularities. The upper spindle has 17 chromosomes at one pole and 18 at the other, while the lower spindle shows 17 and 19 chromosomes with an extrusion at the right of the figure. Figure 55 shows a single laggard at anaphase.

The tetrads are ordinarily regular, but the slight irregularities already referred to indicate that this is not always the case. Figure 56 illustrates a polycaric condition in which one micronucleus is present in addition to the four nuclei expected. At the lower side of the figure a strand of chromatin connects two of the nuclei. A similar strand was observed in several tetrads.

The pollen grains are morphologically perfect in about 95 per cent of the cases, although a few empty grains are to be found. The

grains are practically the same size throughout, although the abnormalities referred to suggest that some grains may contain 17 or 19 chromosomes instead of the true haploid number of 18.

Apomictic development has been noted and will be reported upon more fully later.

Discussion

FORMATION OF POLYPLOID GAMETES

The writer's investigation, as well as those discussed here, shows that through abnormal meiotic behavior pollen grains are often produced which vary markedly from the expected haploid chromosome number. These may be designated as polyploid grains, and the gametes which are formed may be called polyploid also. Such grains may be formed in several ways, as follows.

IRREGULAR DISTRIBUTION OF CHROMOSOMES.—The failure of some or all of the chromosomes to pair at diakinesis leads to the presence of either univalent chromosomes or of both univalent and bivalent chromosomes on the spindle. Under such conditions the equatorial plates may be very irregular, with the chromosomes widely scattered on the spindle. Lagging is often prevalent at anaphase. The varying number of bivalents and univalents and the lagging result in an unequal distribution to the poles. The variation in number is increased by the fact of extrusion. Similar irregularities in the homoeotypic divisions add to the possibilities of an irregular distribution of chromosomes to the daughter nuclei. Polycary, polyspory, and the varying size of the pollen grains indicate that the variation in chromosome numbers may be wide.

HICKS (10) in the Cyperaceae, WOODWORTH (35) in *Betula*, and CHURCH (5) in the Gramineae support this conception with considerable evidence.

NON-REDUCTION.—WOODWORTH (35) described non-reduction in *Betula*. ROSENBERG (29) observed this type of division in *Hieracium pseudoillyricum*, and commented upon the somatic appearance of the chromosomes. Each cell of the dyad produced contains the somatic number of chromosomes for the species. *H. murorum* and *H. smolandicum*, as already shown, carry out this type of division. A summary of these investigations indicates that pollen grains with the

somatic number of chromosomes may be produced in known and suspected hybrids by a process of non-reduction.

GIANT POLLEN GRAINS.—Giant pollen grains have been found in several species in widely separated taxonomic groups. CANNON (3), investigating hybrid cotton, ROGERS (26) and CASTETTER (4), studying *Melilotus alba*, TAYLOR (34) in *Acer rubrum*, GAINES and AASE (8) in *Triticum*, and WOODWORTH (35) in *Betula*, have reported the formation of such grains. In each case hybridization is believed to have been responsible for this abnormal condition. MATSUDA (18) found that forms of *Pelunia*, probably of hybrid origin, produced giant pollen grains which germinated in artificial culture. The anthers of *Hieracium flagellare* contain a considerable proportion of giant grains apparently developed from entire mother cells. It is clear that giant grains have been observed in a number of cases in known and suspected hybrid forms and that such grains may be viable.

SEMIHETEROTYPIC DIVISION.—ROSENBERG (29) described and figured some unusual departures from the normal meiotic process in *Hieracium*. The heterotypic phase showed no normal plates, but the chromosomes were widely scattered on the spindle. At a stage corresponding to anaphase, the chromosomes were still widely scattered on the spindle as laggards. These were so numerous in many instances that the nuclear membranes normally laid down at the poles, instead of cutting through the spindle figure, inclosed the entire chromosome complement in a single nuclear membrane. The single nucleus thus formed was often dumbbell-shaped and gave the appearance of an amitotic division. The constriction was carried even farther in some cells. In many instances, however, the nucleus was rounded out to a normal shape although it contained the somatic number of chromosomes. Such nuclei correspond to interkinetic nuclei, and have become known as restitution nuclei. Those which are of normal shape undergo a division; each chromosome splits longitudinally and the following division is regular, resulting in a dyad of nuclei each of which is diploid for the species concerned.

A number of earlier cytologists have described and figured abnormal meiotic divisions which were probably semiheterotypic, although not recognized by them as such. More recent investigators,

such as BUXTON and NEWTON (2) in *Digitalis* hybrids, KARPECHENKO (13) in *Raphanus* \times *Brassica*, and WOODWORTH (35) in the hybrid *Betula sandbergi*, have recognized this type of division, have described it much as ROSENBERG did, and have applied the term semiheterotypic to it.

The semiheterotypic division is widespread in its occurrence in known and suspected hybrids. It results in polyploid pollen grains which are usually diploid for the species. Slight variations in the process, as described under *Hieracium canadense*, show that in some cases aneuploid dyads are produced.

Polyploid pollen grains may be formed in a number of ways through abnormalities in meiosis. Partial or non-pairing at diakinesis, the presence of both bivalent and univalent chromosomes, lagging, and extrusion, all result in the irregular distribution of chromosomes to the daughter nuclei, so that pollen grains may contain chromosome numbers which vary considerably from the haploid number. Non-reduction, the formation of giant grains, and the semiheterotypic division are other means by which pollen grains containing an abnormal number of chromosomes may be formed. Such grains usually contain the higher polyploid series of numbers.

ORIGIN OF POLYPLOIDY

If it can be demonstrated that polyploid pollen grains do produce viable gametes, a reasonable explanation for the origin of polyploid forms is at hand. Gametes with unlike chromosome numbers may be produced by plants of the same species, or even by the same plant, and these may unite to form polyploid zygotes. Similarly, species of a polyploid series produce gametes with unlike chromosome numbers, and these may unite through interspecific hybridization and produce forms with chromosome numbers differing from those of the parents.

OSTENFELD (23-25) investigated *Hieracium* experimentally, and ROSENBERG (29) examined the resulting hybrids. Referring to hybrids between tetraploid species, themselves regarded as being of hybrid origin, ROSENBERG says, "Researches on the hybrids concerned have shown that, in fact, the varied chromosome number has not influenced the ability to germinate." Hybrids were also pro-

duced between diploid and tetraploid species (the latter probably hybrid). The F_1 generation showed plants exhibiting an astonishing heterogeneity and displaying varied chromosome numbers. Polyploid gametes are thus seen to be viable and to produce types which may form the basis for a new species.

LJÜNGDAHL (15) produced a stable hexaploid type in *Papaver* by crossing *P. striatocarpum* with *P. nudicaule*. The former has a haploid chromosome number of 36 and the latter one of seven. The meiotic divisions of the hybrid exhibited 21 bivalents. Two forms with a tremendous difference in chromosome numbers have been hybridized to form a type with a chromosome number differing from that of either parent. Gametes with widely differing chromosome numbers are thus capable of producing a functional zygote.

The investigations of KARPECHENKO (12, 13) on *Raphanus* \times *Brassica* yielded significant results. Irregularities of meiosis in the hybrid caused the formation of polyploid gametes with from six to 12 chromosomes, or, by a semiheterotypic division, diploid, hypodiploid, and hyperdiploid and tetraploid cells were formed. The F_1 generation produced tetraploid, hypotetraploid, and hypo-hexaploid plants. The F_1 back-crossed to *Raphanus* yielded triploid, pentaploid, and hypopentaploid forms. KARPECHENKO says, "Here we have evidently to deal with only progeny of gametes with nine, 18, and 36 chromosomes or of such approaching the above mentioned ones in number of chromosomes." The hybridization of two species with like chromosome numbers has led to irregularities in meiosis, resulting in the formation of polyploid gametes which have been shown to be viable in various combinations and to have produced plants with a wide range of chromosome numbers. Referring to the tetraploids KARPECHENKO says, "They distinguish themselves by quite normal development, by regular meiosis; they have normal pollen and most of them are fertile. After several generations they will probably all be fertile, as progeny from the fertile plants will increase more rapidly than progeny from plants of reduced fertility, and will displace the latter. When comparing it with the parent species *Raphanus sativus* and *Brassica oleracea*, tetraploids represent a different chromosome number. They appear most difficult to cross with *Raphanus* or *Brassica*, i.e., we observe in them the occurrence of the

sexual individualization to which BATESON (1914, 1922) attached special importance for determining a 'specific nature.' "

The *Crepis* hybrids of COLLINS and MANN (6) have been extensively investigated. *Crepis selosa* with a haploid number of four and *C. biennis* with a haploid number of 20 were crossed. The F_1 meiotic divisions showed 10 bivalents to be present ordinarily. These probably represent pairings of the 20 *biennis* chromosomes, as *selosa* is shown in other cases not to exhibit pairing among its chromosomes. The gametes contained from 10 to 14 chromosomes. The F_2 individuals back-crossed to *biennis* were found to contain 32 chromosomes. The reduction divisions showed 15 bivalents to be present and reduction was almost normal. COLLINS and MANN consider an F_4 plant sufficiently distinct to call it *C. artificialis*. *Crepis* contains a long series of aneuploid species which they believe to have arisen through hybridization.

Experimental work on the production of polyploids has been carried out by NEWTON and DARLINGTON (21). Triploid plants of *Tulipa* have been produced by hybridization of distinct diploid and tetraploid species, as well as by diploid and tetraploid individuals of the same species. Investigation of *Hyacinthus* (NEWTON and DARLINGTON 21) has led to the following statement: "Perhaps correlated with the ordinary fertility of these aneuploid hyacinths is the fact that hyacinths appear to be equally successful with every chromosome number between 16 and 30, so that gametes with very irregular chromosome numbers are no less likely to produce viable zygotes."

NAWASHIN (19, 20) demonstrated the viability of gametes with chromosome numbers other than the haploid number. Triploids of the ordinary *Crepis capillaris*, *C. dioscorides*, and *C. tectorum* appeared in his cultures. The origin is unknown. Seed produced by the open pollination of *C. capillaris* yielded 105 plants. Open pollination of the F_1 gave an F_2 containing every chromosome number from diploid to tetraploid, and in addition a heptaploid plant was found. The triploid plants of *C. capillaris* used as female were crossed with normal plants of various species of *Crepis*. Most of these hybrids showed a diploid complex from *capillaris* and a haploid complex from the other species. Hybrid offspring were also obtained from crosses which had never been successful with diploid *capillaris*.

NAWASHIN states, "these polyploid hybrids of various kinds proved to be much more fertile than the normal diploid ones."

In addition to such experimental studies, there have been many cytological studies on polyploid genera without the background of closely correlated experimental work. Such investigations have had, in many cases, a considerable amount of taxonomic or genetical evidence in support of the cytological results. Among studies of this type are those of TÄCKHOLM (33) on *Rosa*; LONGLEY (16, 17) on *Rubus* and *Crataegus*; AFZELIUS (1) on *Senecio*; HÅKANSON (9) and HICKS (10) on *Scirpus*; and WOODWORTH (35) on *Betula*. These investigators have expressed the opinion that the polyploid series of the investigated genera have arisen through hybridization.

The results of the controlled experimental work and of an examination of natural polyploid series lead to the same conclusions. Polyploid gametes have been produced as a result of hybridization. These gametes have proved viable in various combinations and have resulted in the formation of polyploid hybrid forms. These hybrids intercrossed or back-crossed have led to the establishment of stable types. Such stable types exhibit a different chromosome number from either of the parents and the reduction divisions are regular or nearly so. Certain of these polyploid forms are more fertile than the diploid forms. Some of the types produced have been sufficiently different from any known form to cause a new specific name to be applied to them. The conclusion has been reached that both aneuploid and euploid forms arise through hybridization.

In view of this situation, the statement by DAVIS (7) seems particularly appropriate: "There is coming to be recognized a type of hybrid that reproduces itself perfectly, throwing at most only occasional variants. Such hybrids satisfy fully our concept of species as a kind of animal or plant which breeds true."

Summary

1. Cytological examination of the male meiotic divisions of *Hieracium* yields the following results:

a. *Hieracium venosum*, *H. paniculatum*, and *H. scabrum* of the subgenus *Stenotheca* are characterized by a diploid chromosome number of 18 and exhibit perfectly normal reduction divisions.

b. *Hieracium canadense*, *H. murorum*, and *H. smolandicum* of the subgenus *Euhieracium* are triploid species. Various degrees and combinations of non-pairing, lagging, extrusion, semiheterotypic divisions, polycary, polyspory, and pollen sterility are found in these species.

c. *Hieracium pratense* of the subgenus *Pilosella* is a tetraploid species and reveals lagging and extrusion in the meiotic divisions.

d. *Hieracium flagellare* is a polyploid species, and the reduction divisions show irregularities such as incomplete pairing, lagging, extrusion, polycary, polyspory, and a marked difference in the size of the pollen grains.

2. The species of *Euhieracium* and *Pilosella* exhibit the cytological peculiarities of known hybrids, as well as being polyploid.

3. The species of *Euhieracium* and *Pilosella* develop embryos apomictically.

4. The presence of taxonomic difficulty in the subgenera *Pilosella* and *Euhieracium*, together with the facts of irregular meiosis, polyploidy, and the apomictic development of embryos, is considered as evidence for the hybrid origin of the species of the subgenera here discussed.

5. Hybridism is probably one of the ways by which polyploidy arises, as well as one of the ways by which a multiplication of species is brought about.

This investigation has been carried out under the supervision of Professor E. C. JEFFREY to whom I am greatly indebted for interest and advice.

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STUDY OF THE LIFE HISTORY OF BRASSICA OLERACEA

OSCAR H. PEARSON

(WITH SIXTEEN FIGURES)

Introduction

No detailed morphological study has apparently been made of the flower of *Brassica oleracea* L. This paper reports such a study, and the data apply equally well to all the varieties of the species,—cabbage, cauliflower, brussels sprouts, kohlrabi, kale, broccoli, and sprouting broccoli.

The work on controlled pollination was done at Berkeley, California, during the springs of 1926 and 1930; and the physiological studies on pollen were made at Davis, California, during the winter of 1930–1931. The plants used were cauliflower, broccoli, and cabbage at Berkeley, and sprouting broccoli, grown in the greenhouse, at Davis. The rates of growth and development were secured at Berkeley and Davis, and may only approximate those under other climatic conditions.

Floral development and pollination

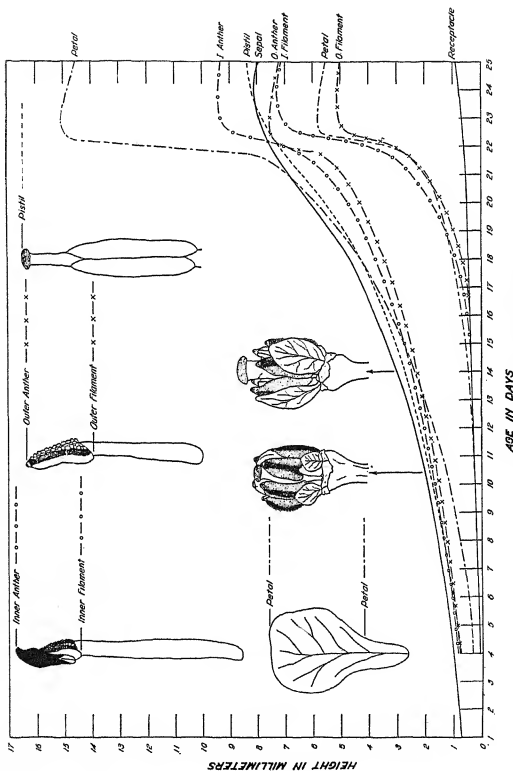
The inflorescence is typically racemose, sometimes paniculate. In cauliflower types, however, the inflorescence is abortive. According to LUND and KJAERSKOV (8), this abortion results from a suppression of the terminal growing point which does not at first affect the lower branches. These lower branches grow to about the same level as the terminal growing point, and are in their turn inhibited. In this way a large "curd" is formed, the blanching and fleshy development of the peduncles being caused by their growing in the shade of the wrapper leaves. Later in the season the side and lower branches resume growth, followed by the more nearly terminal ones. The flowers of the cauliflower are sometimes borne in simple racemes; but usually the inflorescence is made more complex by the lack of divergence of several small peduncles for a considerable distance and by the appearance of only a few flowers on shortened racemes.

The smoothed curves in figure 1 have been constructed to represent the typical development of the floral organs of *Brassica oleracea*. The data for this chart were secured by measuring the outer sepal of the same bud daily from as early a period in its development as it could be distinguished. These measurements fixed the curve for the sepals. More than 100 buds were measured in this way. The curves for the buds which developed normally were of the same shape in all cases, although the rate of growth depended somewhat upon the prevailing temperature. A number of buds of various ages were dissected, and the various floral organs measured to secure data for the curves of development of these organs. These curves differ appreciably from those for *Cardamine pratensis* as shown by GUNT-HART (5), for they show a sudden expansion of the floral parts, as is seen in figure 1. Anthesis in this case was completed in a single day, the twenty-first day after the flower buds were large enough to measure, as shown on the chart.

The method of origin of the floral parts in Cruciferae was described by EICHLER (4). The outer sepal of the lower whorl opposite the raceme axis is first differentiated. The other organs appear in the usual order of petals, stamens, and carpels. Figure 2 shows a longitudinal section through the tip of a raceme, and the mode of origin of the floral organs.

Two theories have been advanced to describe the ovary in Cruciferae. The generally accepted theory considers that it is made up of two carpels; the other that it is made up of four. SAUNDERS (13) has advanced the idea of polymorphic carpels; the valves being thought to be the sterile carpels, whose margins are adnate to two solid, fertile carpels that are prolonged to form the style and stigma. EAMES and WILSON (3) are in general agreement with this theory, but EAMES (2) considers as improbable the presence of solid fertile carpels.

Anthesis usually starts about four or five o'clock in the afternoon. The rate of growth of the petals and filaments of the stamens at this time increases rapidly; the pressure from these organs finally forces the sepals apart. During the night the growth of these organs continues, and in the morning the petals are nearly full length. They are tightly rolled unless, during the process of opening, the sepals

FIG. 1.—Rate of development in flower of *Brassica oleracea*

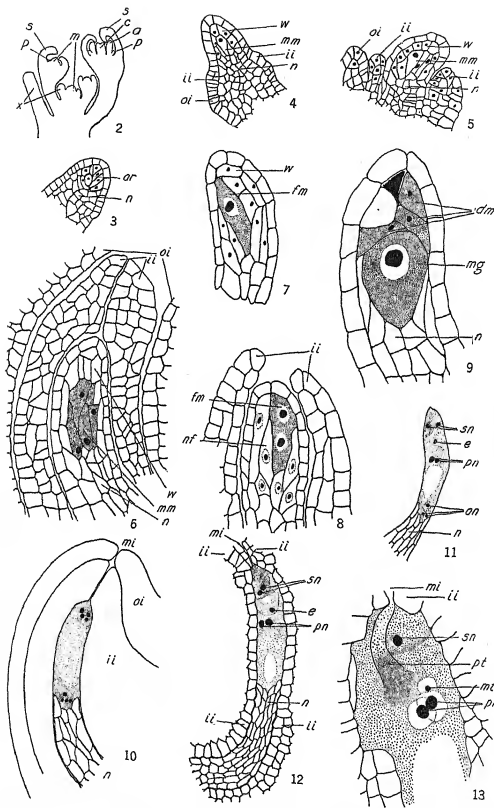
have disarranged them. The flower is usually fully expanded before 10 A.M. Often it opens completely during the afternoon, especially in warm weather. The anthers usually dehisce as soon as the petals begin to reflex.

Brassica oleracea flowers are insect-pollinated. Although ROEMER (12) found that kale and kohlrabi pollen is carried by wind, bees are the chief agents of pollen dispersal. Honey-bees, unlike many of the other insects that visit flowers, do not work at temperatures below 60° F., whereas cabbage blooms most profusely at temperatures which for most of the day are somewhat below that. Bumblebees, although not plentiful in California, are often found on *Brassica* flowers. The solitary bees, closely related to the honey-bee, serve as the chief agents of pollination. Syrphus flies (*Syrphidae*), although occasional visitors to the flowers, are not important pollinating agents. House flies and blow flies are often seen on *Brassica* plants, but they very seldom, except by accident, happen upon either pollen or nectar. HOWARD (6) has described the manner in which honey-bees visit flowers of *B. juncea*.

Nectar is secreted in relatively large amounts by the nectaries between the short stamens and the ovary. It amounts to approximately 0.1 cc. per flower each 24 hours, and is secreted until the third day, when the corolla starts to wither. The nectaries outside the two long stamens are functionless.

Microspore

ONTOGENY.—The archesporial cells are distinguishable in buds as young as the largest shown in figure 2. But three or four layers of wall cells are formed, and the entire development is typical of that found in most angiosperms. Nine bivalent chromosomes are present at the first metaphase of the reduction division. This stage occurs in buds about 1.6–1.8 mm. in length. By the time the buds are 2 mm. long the tetrads have been released from their gelatinous matrix. Further development consists of a growth period, followed by a laying down of the exine as a secretion from the tapetal cells, as described by KOSMATH (7). The first division of the microspore nucleus occurs two or three days before anthesis, and the division of the generative nucleus the day that the flower opens. At the time of shedding there are three nuclei in the pollen grain.



FIGS. 2-13.—Fig. 2, longitudinal section through tip of a *Brassica* raceme; arche-sporial cells visible in anther of largest flower bud; $\times 12.5$. Fig. 3, section through tip of ovule showing cluster of archesporial cells directly beneath epidermis; $\times 200$. Fig. 4, section through tip of ovule showing wall cells just formed and nearly as large as mega-

Ovule and megagametophyte

ONTOGENY.—The primordium of the campylotropous ovule can first be distinguished in buds somewhat less than 1 mm. in length. The integuments appear at the base of the nucellus as two concentric rings of actively growing tissue, just before the reduction division in the pollen mother cells; that is, in buds about 1.5–1.6 mm. in length. At the time the microspores are separate in the tetrads, the integuments have reached the tip of the nucellus. Buds about 5 mm. in length show reduction divisions of the megaspore mother cell. Buds 7 mm. in length show the second and third nuclear divisions of the developing megagametophyte, which is completely organized at anthesis.

The development of the megagametophyte in several genera of the Cruciferae has been described by VANDENDRIES (16). Megagametophytic development in *Brassica oleracea* is similar to VANDENDRIES' description for *Sisymbrium taraxacifolium*. The tip of the nucellus contains several archesporial cells directly beneath the epidermis (fig. 3); these divide to form the primary wall cells and the megaspore mother cells (figs. 4–6). One of the several megaspore mother

spore mother cells just beneath; integuments just beginning to appear; $\times 200$. Fig. 5, section through tip of ovule showing wall cells and megaspore mother cells; cell on left apparently beginning to encroach on other mother cells; $\times 200$. Fig. 6, archesporial cells in tip of ovule; wall cells formed but megaspore mother cell not yet encroaching upon other cells; $\times 440$. Fig. 7, section of nucellus showing wall cells and enlarging megaspore mother cell; larger cells beside the large mother cell are also megaspore mother cells, but not functional; $\times 510$. Fig. 8, result of first division of megaspore mother cell nucleus; partitioning cell wall not yet formed; $\times 510$. Fig. 9, one-celled megagametophyte from bud 5.5 mm. in total length; three anterior daughter cells disintegrating; $\times 775$. Fig. 10, end of third division of megagametophyte, with four nuclei at each end of the enlarged cavity; $\times 290$. Fig. 11, megagametophyte just organized with antipodals present; $\times 220$. Fig. 12, fully mature megagametophyte in stage usually found in fully open flowers; antipodal cells entirely disappeared; central core of thin-walled cells is remains of nucellus; cells bordering them and the megagametophyte are epidermal cells of inner integument; $\times 290$. Fig. 13, triple fusion of polar nuclei and sperm, discharged contents of pollen tube completely hiding egg and sperm; $\times 775$. *a*, anther; *an*, antipodal nuclei; *ar*, archesporial cells; *c*, carpel; *dm*, degenerating megaspores; *e*, egg; *fm*, functional megaspore mother cell; *ii*, inner integument; *m*, meristem; *mg*, megagametophyte; *mi*, micropyle; *mm*, megaspore mother cells; *mt*, microgamete; *n*, nucellus; *nf*, non-functional megaspore mother cells; *oi*, outer integument; *p*, petal; *pn*, polar nuclei; *pt*, pollen tube; *s*, sepal; *sn* synergids; *w*, wall cells; *x*, pedicels of buds not shown in section.

cells, usually that near the center of the nucellus, continues to develop, the others remaining passive and being digested or pushed aside by the enlarging megaspore mother cell (fig. 7). The wall cells do not develop further, and are absorbed or pushed aside by the enlarging megaspore. By two successive divisions a linear tetrad is formed (fig. 8); the chalazal megaspore becomes very large; while the three micropylar spores remain small and soon degenerate (fig. 9). These cells are digested and absorbed until finally the one remaining megaspore occupies the entire tip of the nucellus, the parietal cells having been pushed aside or digested. The megaspore nucleus undergoes three successive divisions, and the eight nuclei (fig. 10) become arranged as shown in figure 11. At this time the upper end of the megagametophyte lies in contact with the inner integument, the apical portion of the nucellus having been digested. The antipodals disappear early.

At the center of the ovule there is a distinct core of thin-walled, long, loose cells, light-staining and apparently dead or dying (fig. 12), the remains of the nucellus which extend toward the chalaza from the proximal end of the megagametophyte. These gradually disappear as the embryo sac increases in size after fertilization. VANDENDRIES (16) and RIDDLE (11) describe a similar tissue. The major portion of the ovule at anthesis is therefore composed of the fleshy inner integument.

Microgametophyte

The surface of the stigma is made up of long papillate cells. At anthesis and while the flower is fully expanded, these cells are moist. Between them is a thick gelatinous substance, as seen in fresh sections stained with aceto-carmin. Just below these papillae are several layers of cells, somewhat elongated at right angles to them. This layer, following the curves of the surface of the stigma, is compacted into a core, similar to the usual stylar canal, directly below the commissural cleft. It continues to the base of the ovary as the central tissue of the partition wall or replum. In this tissue the pollen tubes grow. Full expansion of the stigmatic papillae is not necessary for germination of the pollen. Empty pollen grains with traces of pollen tubes between the unexpanded papillae were found in sectioned flowers pollinated before anthesis.

Age of the ovary has apparently little influence on the rate of pollen-tube growth, for in a bud 4.2 mm. in length, pollen tubes were observed half way down the ovarian cavity 10 hours after pollination, or at about the same place they would have reached had the pistil been mature. In this case, since the ovules in this ovary still contained megaspore mother cells, fertilization could not occur for several days. An ovary killed 151 hours after pollination had, at the time of preserving, mature megagametophytes. It had been pollinated six days before fertilization could occur. Dead pollen tubes, much curled and twisted, were observed on the funiculi, but no fertilization stages were seen.

POLLEN TUBE BEHAVIOR.—The following studies were made in controlled pollinations of emasculated broccoli flowers at Berkeley during May, 1926. The flowers were killed in a weak chromacetic solution and imbedded in paraffin; the sections 8–10 μ thick were then stained in Haidenhain's iron-alum haematoxylin. The ends of the tubes stain heavily and can easily be distinguished.

The pollen, after being deposited on the stigma, remains inactive for a short time. At the end of the second hour, tubes may be found extending to the bases of the papillae. The pollen tube often winds and twists on the surface of the stigma. Finally, growing down the side of one papilla, it enters the tissue of the stylar canal, growing between the cells. Because of the deep-staining nature of the contents of the tip of the pollen tube, nothing can be distinguished as to nuclear behavior.

In pollen tubes grown in sucrose solutions, then killed in absolute acetic alcohol and stained in Delafield's haematoxylin and safranin, according to the procedure described by WEBBER (17), the vegetative nucleus is found at the tip of the tube, and the microgametes appear as lighter bodies close together, about 0.05–0.08 mm. from the tip. VANDENDRIES (16), however, found that the microgametes precede the tube nucleus. According to VANDENDRIES, the pollen tube emerges from the replum into the ovarian cavity and grows along the surface until it comes in contact with the funiculus of an ovule. It follows this down to the micropyle and passes between the integuments into the megagametophyte. As soon as it enters, the contents are emptied upon the egg, making observation of fertilization difficult. One sperm, however, can be seen with the polar nu-

clei, the three ready to fuse (fig. 13). Fertilization takes place about 16-20 hours after pollination.

PHYSIOLOGY.—BACH (1) studied the germination of pollen of oil mustard (*Brassica rapa oleifera*) in hanging drops of sucrose solution ranging from 2 to 50 per cent concentration. Best results were secured in 15 to 25 per cent solutions. At room temperature the pollen was viable for four days, but did not remain so for eight days. Pollen in a desiccator in a cellar remained viable for several weeks, but it died in a day or two if stored in the open air of a cellar.

The pollen from flowers of sprouting broccoli plants grown in the greenhouse were used in the tests reported here. Agar smears were found to be unsatisfactory because of lack of regulation of humidity. Van Tieghem cells proved to be the most useful. In 10 per cent sucrose solution pollen grains remained inert; in 15 per cent a high percentage of the resulting pollen tubes ruptured; in 20 per cent the growth was apparently normal, long tubes being formed with only a small percentage of broken ones; in 25 per cent the tubes were not so long, although they appeared very similar to those in the 20 per cent solution. In solutions of glucose, no matter what the concentration, the pollen grains exuded a granular-appearing substance (possibly oil droplets); no pollen tubes developed.

Brassica oleracea pollen germinates over a considerable range of temperature. No germination was secured at temperatures below 10° C., but it was excellent at 15° and 20°; at 26° the tubes were noticeably shorter but apparently normal. At 30°, however, the tubes were short and irregular and almost invariably burst. At 35° only a few grains germinated in this way, the rest remaining inert; at 40° C. no germination whatever was secured. In these tests, pollen was taken from an individual freshly opened anther for any series of trials; and several different trials from the same plant and from different plants were run. The results were always the same.

Results of tests of pollen longevity in a constant temperature chamber showed no difference in germinability of pollen from different anthers of the same flower. For any given date the magnitude of germination was about the same. Both high and low temperatures are injurious to *Brassica* pollen in the dry state. At 4° C. the pollen remained viable two days; at 30° and 35°, only one day; at 11°, six

or seven days. At 21°, however, viability sharply decreased after the second day. Maximum longevity of the pollen, therefore, is found in the vicinity of 11° C. The longevity of pollen under greenhouse conditions (60–85° F.) was likewise tested. The flowers that opened each day upon a raceme were tagged, and germination tests were run at one-day intervals up to and including five days. In no case did pollen more than three days old germinate.

To discover whether these findings are also true for pollen applied to stigmas, pollen of various ages was applied to immature stigmas. Immature stigmas were used to avoid technical difficulties, since it has been shown that young stigmas are receptive (PEARSON 9). The results showed that pollen four days old is a little less likely to grow than fresh pollen, that when five days old it is much weaker, while pollen six days old is impotent. The slightly longer viability in this as compared with the preceding test doubtless resulted from difference in temperature. Unfortunately no tests were conducted to test the influence of humidity upon the viability of pollen. BACH's results (1) with turnip seem to indicate that humidity is at least as important as temperature in determining the length of time for which pollen can be stored.

Embryogeny

RIDDLE (11), SCHAFFNER (14), SOUÈGES (15), and VANDENDRIES (16) give detailed discussions of the embryo and its ontogeny in Cruciferae. Instead of tracing cell lineage, therefore, an attempt has been made to determine and demonstrate the times at which different stages occur.

ENDOSPERM.—Very shortly after triple fusion takes place, the endosperm nucleus divides. One of the daughter nuclei remains near the zygote while the other migrates toward the antipodal end of the embryo sac. Division follows division in rapid succession. Twenty-three hours after pollination, or about 5 hours after fertilization, three endosperm nuclei were found; 56 hours after pollination, 36 nuclei were found. At this time there were usually eight or ten endosperm nuclei in the micropylar neck of the enlarging embryo sac. These may be closely synchronized in development, since two cases of simultaneous division were found.

Many of the remaining endosperm nuclei are found at the periph-

ery of the sac, slowly digesting the adjoining cells of the inner integument. As already pointed out, the nucellus completely disappears shortly after fertilization. A number of very large, deep-staining endosperm nuclei are found at the chalazal end of the embryo sac, imbedded in a heavily staining granular substance. This substance, obviously a mechanism for transfer of the nutritive material for the embryo, can be seen through the walls of the living ovule as a bright green spot about 12 to 15 days after pollination. VANDENDRIES described this formation as the result of a hypertrophy of the cells of that region. A similar substance is found about the suspensor of the embryo.

DEVELOPMENT OF SEED.—The method employed for the study of seed development consisted of heating ovules of known age in a strong solution of KOH, nearly at the boiling point. The length of time varied from 5 to 20 minutes, depending upon the degree of clearing desired. These ovules were then put upon a slide in a drop of the solution, placed under a microscope, and traced with a camera lucida. The rate of growth of the ovule and embryo as described is that found in the material used, under conditions at Davis.

The central core of the ovule at the time of fertilization is divided into two parts: the portion toward the micropyle is the megagametophyte; that toward the chalaza is the thin-walled, nucellar tissue in the process of being absorbed by the developing gametophyte. The development of the viscid greenish substance at the chalazal end previously mentioned is not shown by the means of dissection employed until the ninth day. In prepared slides, however, the beginning of this so-called hypertrophy can be detected as early as the first few divisions of the endosperm nuclei in that region of the sac. The rate at which the seed increases in size is indicated in figure 16. The outer integument is three or four cells in thickness; the portion of the inner integument which resists the dissolving action of the endosperm nuclei is several cells thicker. These cells are modified in several ways in the formation of the seed coats, as described by PIETERS and CHARLES (10). The final change in color of the seed coats does not occur until about 40 days after pollination.

DEVELOPMENT OF OVARY.—Rate of growth of the ovary or silique closely parallels that of the ovule, but not that of the embryo.

Figure 14 shows the rate of growth of three typical siliques of sprouting broccoli, grown in a greenhouse at Davis in January, 1931, from the time the ovary could be distinguished (fig. 1) until growth ceased. Anthesis occurred on the twenty-eighth day. Ovaries *A* and *C* were pollinated at that time, while ovary *B* was not pollinated until the thirty-second day. The rate at which these siliques grew was followed by means of daily measurements. The number of seeds produced by *A* and *B* was 20 and 26 respectively. Silique *C* produced but 13 seeds, a fact correlated with its lesser length. Maximum growth was reached about 20 days after pollination. The ovules reached their maximum size in about 18 days, but the embryos did not reach maturity until about the thirty-third day after pollination. Ovarian growth is likewise shown to be closely associated with development of the ovule by the parallelism between the curves for ovaries *A* and *B*. No change occurred in *B* until pollination; but once growth of the ovule began, growth of the ovary proceeded normally.

Figure 15 shows a dependence of the ovarian growth upon growth of the ovule. The curves shown on this chart are of typical siliques of broccoli measured daily at Berkeley during 1926. There is evidently a close relation between the number of seeds formed and the length of the silique. The rate of increase in length is the same whether there are 3 or 15 ovules fertilized; but when but a small number are present, growth ceases after a period of time roughly proportional to the number of developing seeds. In these curves, as contrasted with figure 14, the maximum length was reached a day or two later, but the temperature was lower than at Davis.

Elongation of the silique often occurs without the formation of seed, especially under conditions of high temperature or of reduced light. Frequently ovules in such siliques are swollen, the ovules being sometimes apparently undeveloped. Such siliques are usually found late in the blooming season, when the maximum temperatures have been above 35° C. for several days.

The photosynthetic cells of the wall of the silique are concerned in the nutrition of the embryo, apparently furnishing a considerable portion of its food supply, since flowers inclosed in manila paper bags after fertilization developed into siliques containing shrunken seed.

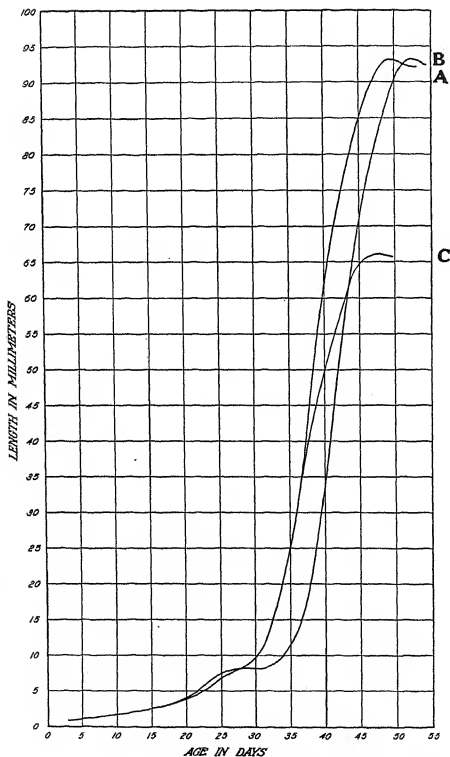


FIG. 14.—Rate of growth of ovary of sprouting broccoli before and after pollination. Ovaries represented by *A* and *C* were pollinated at anthesis; that represented by *B* was not pollinated until four days later.

EMBRYO DEVELOPMENT.—Development of the embryo follows the mode described in other Cruciferae by RIDDLE, SCHAFFNER, SOUÈGES,

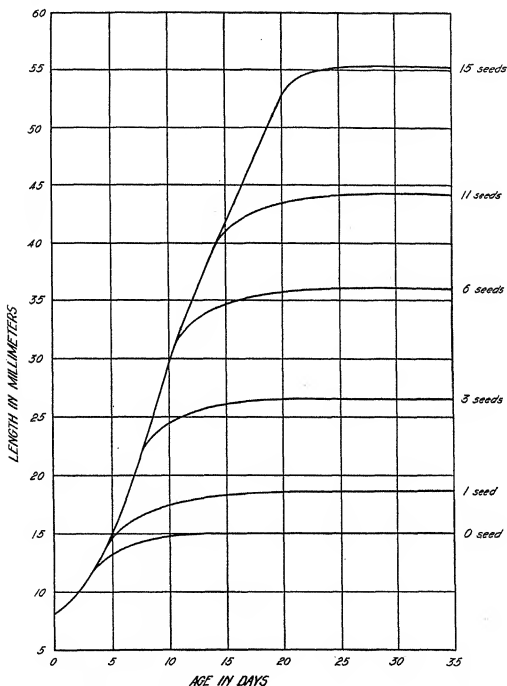


FIG. 15.—Influence of number of seeds upon rate of growth and final length of ovary.

and VANDENDRIES. The zygote does not begin growth until several days after its formation, and until the embryo sac has greatly en-

larged. The first division occurs three or four days after pollination. About the seventh day there are four or five cells in the suspensor and eight cells in the embryo. The cotyledons are differentiated about the thirteenth day. Growth continues in a straight longitudinal direction until about the nineteenth day, when the tip of the embryo strikes the inner wall of the embryo sac. Further growth

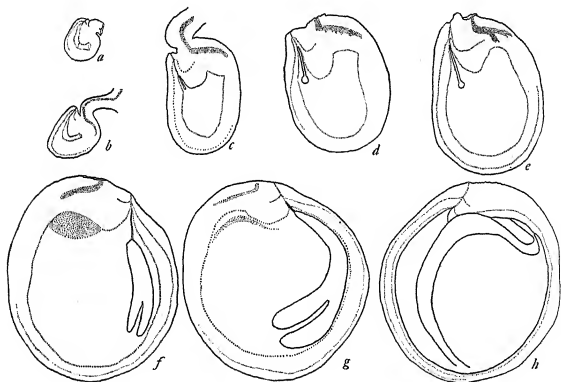


FIG. 16.—Series of drawings, to same scale, of ovules from time of anthesis until embryo is mature; ovule reaches maximum size about 19–20 days after anthesis, if fertilization has taken place. *a*, ovule at anthesis; *b*, ovule after 3 days; *c*, ovule after 7 days; *d*, ovule after 11 days; *e*, ovule after 14 days; *f*, ovule after 19 days; *g*, ovule after 21 days; *h*, ovule after 32 days; $\times 15$.

causes it to become completely curved upon itself; the cotyledons conduplicate. The 7 to 10-celled suspensor in *Brassica oleracea* does not have a swollen basal cell as in *Capsella bursa pastoris* and some other Cruciferae, but resembles that of *Alyssum macrocarpum*. Figure 16 also indicates the rate at which the embryo grows. It reaches maturity 32–35 days after pollination. Ovules produced at Davis were placed in soil 33 days after pollination and 50 per cent of them germinated.

Summary

1. The inflorescence of *Brassica oleracea* is racemose, but in some varieties is modified by lack of divergence to paniculate, as in cauliflower.

2. Anthesis is caused by the rapid growth of petal and filament, which forces apart the sepals, exposing anthers and stigma.

3. Pollination is brought about by insects, chiefly honey-bees and solitary bees in search of pollen, though also gathering nectar.

4. Reduction divisions of the pollen mother cell were found to occur in buds 1.6–1.8 mm. in length. The haploid number of chromosomes is nine. The first division of the microspore nucleus occurs two or three days before anthesis; the second, on the day the flower opens. The vegetative nucleus precedes the microgametes into the tube.

5. Pollen grows readily in 20 per cent sucrose solution at 15°–20° C. The range of temperatures for normal germination is from 10° to 26° C. Pollen is viable about three days at 20° C., but at 30° fails to germinate on the second day, and at 4° fails to germinate on the third day. Good germination was secured after seven days at 11° C. These results were supported by pollination tests in the greenhouse.

6. Several archesporial cells are formed in the ovule. The first division of the megaspore takes place in buds about 5 mm. in length; the second and third in buds about 7 mm. long. Growth of the megagametophyte absorbs or displaces all the distal nucellar tissue, so that the embryo sac lies adjacent to the inner integument. The antipodals disappear soon after the megagametophyte is organized, usually before fertilization.

7. The embryo does not begin development for at least four days after fertilization. The suspensor of a large embryo consists of six to ten cells. Growth of the embryo is slow at first; there are but four cells in the true embryo on the sixth day after pollination. About the thirteenth day the cotyledons appear; about the nineteenth day the embryo becomes curved; and about the thirty-second day the embryo fills the ovule. At Davis, germination was secured from seeds planted and developed within 33 days after pollination.

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CHROMOSOME PAIRING, STRUCTURAL HYBRIDITY, AND FRAGMENTS IN ROSA¹

EILEEN WHITEHEAD ERLANSON

(WITH TWENTY-THREE FIGURES)

Introduction

The chromosomes of dicotyledons are small, and a true picture of their pairing behavior can be obtained only under optimum conditions of fixation, staining, and microscopy. Evidence in support of the chiasma theory of chromosome pairing (DARLINGTON 6) has been accumulated from widely separated genera, so that it is now indisputable that chromosome pairing at the first metaphase of meiosis in animals and plants is conditional upon the formation of chiasmata among the chromatids of homologous parts of chromosomes in prophase of meiosis.

Multiple associations of chromosomes may arise from: (1) the presence of more than two homologous whole chromosomes in a nucleus, as in polyploids and trisomic organisms; (2) the presence of more than two homologous chromosomal segments owing to reduplication in diploids and polyploids; and (3) reciprocal translocation (STURTEVANT and DOBZHANSKY 18), which is also called segmental interchange between non-homologous chromosomes (ERLANSON 12). References to each type of multiple association have been compiled by DARLINGTON (8). Multivalent groups resulting from each of these causes have been found in *Rosa*.

Reciprocal translocation and reduplication

Reciprocal translocation appears to occur rather frequently in diploid roses, and is an important cause of sterility because of the consequent production of non-disjunctional gametes (BEADLE 1, GAIRDNER and DARLINGTON 13). The first rose in which it was ob-

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served was the garden hybrid polyantha rose Orleans (species complex *Rosa multiflora* Thunb.), a plant of which had a low percentage of trivalent, quadrivalent, and sexivalent chromosome associations at diakinesis and at first metaphase in the pollen mother cells (ERLANSON 12). A plant of *R. blanda* Ait. (diploid), with similar configurations, was found to be 3x (triploid) for a short chromosomal segment. Quadrivalent groups have since been observed in other individuals of *R. blanda*, as well as in plants belonging to the related diploid species *R. woodsii* Lindl. and *R. pisocarpa* A. Gray (individuals of wild origin). In no rose examined has there been a regular formation of quadrivalent groups in all pollen mother cells at meiosis. This shows, on the chiasma theory of chromosome pairing, that the translocations and reduplications concerned are short segments; they are consequently only rarely able to form chiasmata at diplotene. An analogous situation has recently been reported in *Zea* by BURNHAM (2) and BEADLE (1), and is thought by MÜNTZING (16) to be present in *Galeopsis*.

The diploid roses that show multivalent configurations may have small segments of some chromosomes reduplicated, but my earlier report of whole chromosome reduplication in *R. blanda* was in error (ERLANSON 10). The mistaken interpretation was due to multivalent configurations in conjunction with the presence of univalents. In *Rosa* the unpaired chromosomes may divide at both anaphases of meiosis and give rise to 8-chromosome gametes ($n=7$), which, however, seem to be non-functional.

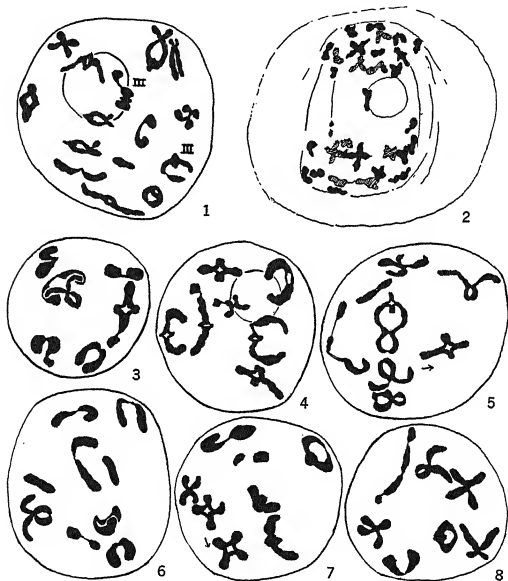
True aneuploidy is very rare in *Rosa*. The two extra chromosomes of the 16-chromosome *R. pyrifera* from Utah (10) are smaller than the others. Their pairing behavior, which is discussed in detail later, shows that they are reduplicated fragments.

An aberrant sterile plant (6424/1)², which was raised from seed of the tetraploid *R. arkansana* Porter, obtained from a nursery, was found to have three extra chromosomes which frequently formed trivalent groups at diakinesis (fig. 1). The first anaphase was irregular, and lagging chromosomes sometimes caused a restitution nucleus to be formed at first telophase (fig. 2).

² Serial accession number in the Botanical Garden of the University of Michigan.

Chromosomes and fragments in *Rosa pyrifera*

A plant of *Rosa pyrifera* Rydberg (6610B) from Utah was found to have 16 pairs of chromosomes (10). One pair was distinctly small-



FIGS. 1-8.*—Fig. 1, diakinesis in atypical offspring of *R. arkansana* with two trivalents, 12 bivalents, and one univalent. Fig. 2, restitution nucleus at first telophase in atypical *R. arkansana* with 31 chromosomes. Figs. 3-8, diakinesis nuclei in *R. pyrifera* (6610B), $2n=14+f+f'$. Figs. 3, 4, f and f' paired with different pairs of chromosomes. Fig. 5, $1_{III}+6_{II}+1'$; f and f' in the trivalent group. Fig. 6, f and f' paired with each other, also $6_{II}+2_I$. Fig. 7, 7_{II} ; f and f' unpaired. Fig. 8, f and f' paired with different members of the same pair, also 6_{II} .

* Figures drawn with a camera lucida at a magnification of approximately 6000 and reduced to two-thirds in reproduction; except figs. 9-12 and fig. 22, which were drawn at approximately 4500 \times and reduced to three-fourths in reproduction.

er than the other seven. This plant is not entirely hardy in Michigan and produces only a few flower buds. Some young anthers fixed in acetic alcohol were cut at $20\ \mu$ and stained with Newton's gentian violet method. A sufficient number of whole nuclei at diakinesis were obtained for a detailed study of chromosome pairing at this stage. Although very few nuclei have been observed at earlier or later stages of meiosis, the conditions at diakinesis are similar to those in other roses at the same stage, and are therefore believed to give a reliable idea of the chromosome organization in this plant.

TABLE I
ROSA PYRIFERA (6610/B), $7_{II}+f+f'$; ANALYSIS OF CHIASMATA
AT LATE DIAKINESIS

NO. OF DIVISIONS OBSERVED	NO. OF POTENTIAL PAIRS OF CHROMOSOMES	TOTAL NO. OF XTA	NO. OF XTA TERMINAL	MEAN NO. OF XTA PER BIVALENT	TERMINAL- IZATION COEFFICIENT*	HIGHEST NO. OF XTA IN ANY PAIR
25	175	221	127	1.27	0.57	3

* Proportion of total chiasmata terminal at any stage (13).

The two small extra chromosomes in the rose 6610B pair not only with each other, but also with two different pairs among the normal set of fourteen. Only homologous parts of chromosomes pair. To interpret chromosome behavior we must assume homologies that are in accordance both with the parts capable of pairing and with the frequencies of pairing. The members of the small extra pair are unequal. They must evidently be considered as fragments rather than as whole chromosomes, since the larger of the two is about half as long as the smallest of the normal chromosomes. The larger is here designated as fragment f' ; the other, slightly smaller, is called f .

Chiasma formation was analyzed in 25 whole nuclei at diakinesis in the pollen mother cells (figs. 3-8). As shown in table I, the mean number of chiasmata per bivalent is only 1.27, calculated for the seven normal pairs. Table II gives the frequencies of the same chiasmata (from 1 to 3) in bivalents (in uncut nuclei). As in other roses, there is a failure of chiasma formation in one pair of chromosomes in a small number of nuclei. In table III the individual 6610B of *R. pyrifera* is compared in its chromosome behavior at late diakinesis with the other two roses (12) in which chiasma formation has been

studied. The *R. pyrifera* plant has a slightly lower chiasma frequency than the other two, which is perhaps due to terminalization having proceeded further. The percentage of bivalents lacking chiasmata is similar in all three, yet the *R. pyrifera* has only one chiasma in 70.3 per cent of the bivalents. This is a higher proportion than was shown even at first metaphase by the other two roses. The terminalization coefficient is higher in this rose. The fragments fail to

TABLE II
CHIASMA FREQUENCY FOR 14 WHOLE CHROMOSOMES IN
ROSA PYRIFERA AT LATE DIAKINESIS

	NO. OF CHIASMATA				TOTAL POTENTIAL BIVALENTS	TOTAL CHIASMATA	MEAN NO. OF XTA PER BIVALENT
	0	1	2	3			
No. of bivalents	4	123	46	2	175	221
Percentage of bivalents	2.3	70.3	26.3	1.1
Total no. of chiasmata observed	0	123	92	6	175	221	1.27

TABLE III
COMPARISON OF CHROMOSOME BEHAVIOR IN THREE DIPLOID
ROSE INDIVIDUALS AT LATE DIAKINESIS

NAME OF ROSE	PERCENTAGE OF BIVALENTS FAILING TO PAIR	MEAN NO. OF XTA PER BIVALENT	TERMINAL- IZATION COEFFICIENT	PERCENTAGE OF BIVALENTS WITH ONLY ONE XMA	HIGHEST NO. OF XTA OB- SERVED IN ANY BIVALENT
Rose Orleans	2.6	1.65	0.50	33.12	3
Rosa blanda, 7N29	1.3	1.47	0.47	52.6	3
Rosa pyrifera, B.	2.3	1.27	0.57	70.3	3

pair more frequently than the major chromosomes, as would be predicted on the chiasma theory. In the nuclei studied they have been paired by terminal chiasmata only. As in *Fritillaria imperialis* (DARLINGTON 5), all the chiasmata are near the ends of the fragments, and terminalization is complete at diakinesis in these though not in the longer chromosomes.

To analyze the pairing behavior of fragments, each of them has to be treated as a potential half-bivalent; where they form a chiasma

with each other, or with a chromosome, the individual fragment is credited with a half chiasma (5, 12). Table IV shows that the shorter fragment, *f*, has a higher pairing frequency than its longer mate, *f'*. The mean number of half chiasmata for *f* is 1.1, which is a little less than half the mean for each chromosome of the normal set (2.54); while the mean chiasma frequency of *f'* is almost a third less than

TABLE IV
PAIRING OF FRAGMENTS IN 25 NUCLEI IN *ROSA PYRIFERA* (6610B)

No. OF 1/2 CHIASMATA	0	1	2	TOTAL NO. OF 1/2 CHIASMATA	MEAN NO. OF 1/2 XTA PER FRAGMENT	No. OF 1/2 XTA TERMINAL	TERMINAL- IZATION COEFFI- CIENT
Fragment <i>f</i>	1	20	4	28	1.1	28	1
Fragment <i>f'</i>	3	22	0	22	0.88	22	1

TABLE V
TYPES OF PAIRING BETWEEN FRAGMENTS *F* AND
F' AND CHROMOSOME PAIRS *AA* AND *BB*

PAIRING ARRANGEMENT	No. OF NUCLEI
1. <i>AA</i> + <i>f</i> , <i>BB</i> + <i>f'</i>	12
2. <i>AA</i> + <i>f</i> , <i>B</i> + <i>f'</i>	1
3. <i>A</i> + <i>f</i> + <i>A</i> , <i>BB</i> + <i>f'</i>	1
4. <i>A</i> + <i>f</i> + <i>A</i> , <i>f'</i> free.....	1
5. <i>AA</i> + <i>f</i> , <i>f'</i> free.....	1
6. <i>f</i> + <i>f'</i>	5
7. <i>AA</i> + <i>f</i> + <i>f'</i>	1
8. <i>A</i> + <i>f</i> + <i>f'</i>	1
9. <i>f</i> + <i>AA</i> + <i>f'</i>	1
10. <i>f</i> and <i>f'</i> free.....	1
Total nuclei.....	25

that of the other chromosomes. In 25 nuclei, fragment *f'* was free three times, while *f* was free only once. The reason for this is shown in an analysis of the types of pairing undergone by the fragments (table V). The small fragment, *f*, pairs with the smallest pair of chromosomes (figs. 3, 4), which I have designated as *AA*, and *f'* pairs with a medium sized pair, *BB*. The pairing arrangements 1-5, table V, are very similar; the fragments are paired with different whole pairs. Since the homologies of chromosomes are specific to

their parts, the constitution of the parts involved in these pairing arrangements can be represented thus: $AA = \frac{ab}{ab}$, $f = b$, $BB = \frac{cd}{cd}$, $f' = c$. Arrangements 6, 7, 8, and 9, table V, show that f and f' also pair with each other and with the same pair, AA (figs. 5, 6, 8); therefore one of them consists of two segments. We may assume that this is the larger f' , because it has the lower pairing frequency. The constitutions of the chromosomes and fragments involved are therefore $\frac{ab}{ab}$, b , $\frac{cd}{cd}$, cb .

In prophase, f' would have difficulty in using both ends at once, and this discontinuity in pairing homology reduces its chiasma frequency (DOBZHANSKY 9).

The pairing behavior of these fragments shows that the plant is quadrivalent for a short segment (b above), and trivalent for another small segment (c above). DARLINGTON (NEWTON and DARLINGTON 17) has pointed out that short fragments are better testers of homologies because they are not carried away by long continuities of homology. Because f' consists of two segments, cb , both fragmentation and translocation must have taken place. The configura-

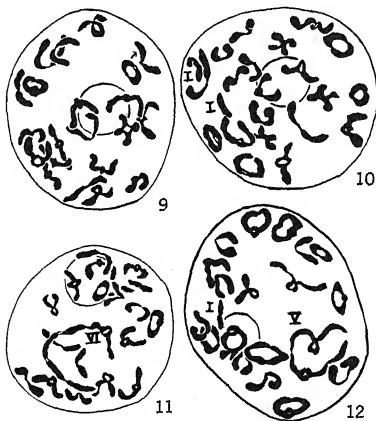
tion $\frac{ab}{ab} + b + bc + \frac{cd}{cd}$ has not been observed; it would be expected to occur infrequently if the translocation c be rather short. Changes of homology arrest terminalization (12), and it is possible that when they are present the process of terminalization causes a strain which might cause fragmentation; but in *Rosa* and *Oenothera* (DARLINGTON 7) such arrest does not lead to breakage of chromatids. There is no evidence to indicate that translocations tend to occur at the same locus in particular chromosomes (2).

This is the only instance of fragments that has been found in *Rosa*. DARLINGTON (3, 4) predicted that effective fragmentation would be rare in the great plant genera *Rubus*, *Ribes*, *Rosa*, *Prunus*, *Avena*, and *Triticum*. Fragmentation is not absent, but when it occurs it is usually eliminated in the course of sexual reproduction. It may become fixed by apomixis, however, as in *Potentilla* (MÜNTZING 15). This plant of *R. pyrifera* has 40 per cent of the pollen grains either dwarfed or shriveled.

Rosa pyrifera belongs to the species complex of *R. woodsii*, from which it differs chiefly in having pyriform hips and pubescent foliage.

Pairing in higher polyploids

Multiple chromosome association occurs in all the balanced tetraploid, hexaploid, and octoploid roses I have examined. Tetraploids frequently show one or two quadrivalent groups in 50 per cent or more



FIGS. 9-12.—Diakinesis in F_1 of *R. engelmanni* \times *nulkana*, $2n=42$; nuclei with $21II$, $20II+2I$, $1VI+18II$, and $1V+18II+1I$ respectively.

of the nuclei at diakinesis and first metaphase (10, 12). Non-disjunction is frequently associated with rings and chains of chromosomes, and this is probably one of the causes of the relatively high sterility found in tetraploid species of *Rosa* (11), as in autopolyploids generally (8). Multivalent chromosome groups are less frequent in polyploids higher than tetraploid. This may be due to a difficulty in obtaining maximum association for all pairing possibilities among sexivalent homologous segments in prophase, on a basis of random chi-

asma formation in these short chromosomes (13). In hexaploids there are 21 pairs in a majority of nuclei at diakinesis (fig. 9); occasionally $20_{II}+2_I$ appear (fig. 10). More than one chain of five or six has not been observed in one nucleus (figs. 11, 12), although as many as four quadrivalent groups have been found (ERLANSON 10, fig. 63).

Figures 9-12 are diakinesis configurations in an F_1 individual from *R. engelmanni* (6x) \times *nuttiana* (6x). The male parent was obtained from the Priest River region of northern Idaho, and the female parent from the Medicine Bow Mountains of Wyoming. The offspring are hexaploid, have only 10 per cent of the pollen empty, set good fruit, and would be classified as *R. engelmanni* by a taxonomist.

The multivalent configurations considered in this section are presumably due to the presence of more than two homologs in the nuclei of the polyploids. If reciprocal translocations are present and are of sufficient dimensions to form chiasmata, then associations of more than four chromosomes appear in tetraploids, as in *R. relicta* (12). Associations of more than six chromosomes have not been found in hexaploids.

Chromosome pairing in unbalanced polyploids

TRIPOIDS.—The first wild triploid roses that I reported (10) were spontaneous hybrids between diploid and tetraploid species. They usually had seven paired and seven univalent chromosomes at diakinesis. A triploid has since been found in a culture of diploid *R. blanda*, from Michigan. This plant probably originated in the fusion of a diploid with a normal haploid gamete of the same species. It had a high percentage of trivalent groups at diakinesis and first metaphase of meiosis; different nuclei showed from three to seven trivalent groups. This variation is characteristic of non-hybrid triploids, and is expected on the chiasma theory of pairing (8). Figure 13 shows a first metaphase with five trivalents and two univalents. Figure 14 shows the end of first anaphase with six chromosomes at one pole, eleven at the other; one pair lagging and two univalents dividing at the equator.

In a culture of diploid *R. macounii* Greene (species complex *R. woodsii*) from Reno, Nevada (no. 12205/15), which was grown from seed of a single individual and cultivated at the California Institute

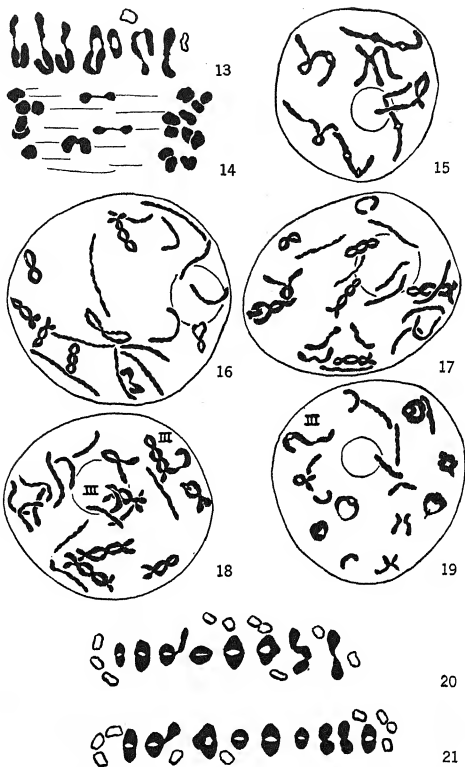
of Technology, one plant among 21 was found to have 90 per cent of the pollen shriveled. Cytological examination showed that it was a triploid with a high proportion of trivalents at diakinesis. Among ten whole nuclei, three had seven trivalents (fig. 15), five had $6_{III} + 1_{II} + 1_{I_1}$, and two had $5_{III} + 2_{II} + 2_{I_1}$. This plant also probably arose from an unreduced gamete.

A seedling of *R. pisocarpa* Gray (12259) with sterile pollen and ovules was also triploid with 21 somatic chromosomes, and had a high percentage of trivalents at diakinesis. The parent plant grew near Jackson, Oregon, and was laden with fruit; it was presumably a normal diploid.

TABLE VI
TYPES OF PAIRING IN 20 NUCLEI OF *ROSA VILLOSA*

PAIRING ARRANGEMENTS	NUCLEI AT EARLY DIAKINESIS	NUCLEI AT LATE DIAKINESIS	NUCLEI AT METAPHASE
$7_{II} + 14_{I_1}$	1	3	1
$1_{III} + 7_{II} + 11_{I_1}$	1	3	1
$1_{III} + 6_{II} + 13_{I_1}$	1
$2_{III} + 5_{II} + 12_{I_1}$	1
$2_{III} + 6_{II} + 10_{I_1}$	1
$8_{II} + 12_{I_1}$	6
$9_{II} + 10_{I_1}$	1

A TETRAPLOID IN SECTION CANINAE.—Chromosome pairing was analyzed in a few whole nuclei of the unbalanced tetraploid *R. villosa* L. This rose belongs to the section Caninae, all the members of which have been reported by TÄCKHOLM, HURST, HARRISON, and BLACKBURN to be unique in never having more than seven bivalents at diakinesis and first metaphase. On the chiasma theory of chromosome pairing, one would expect occasional variations in this arrangement owing to the presence of more than two homologous chromosomes. Table VI shows the types of chromosome pairing actually found in 20 nuclei at early and late diakinesis and at first metaphase. The chromosomes are small and it is difficult to analyze pairing conditions, especially at early diakinesis and metaphase. These few figures, however, show clearly that $7_{II} + 14_{I_1}$ appear only in a minority of the nuclei. In these 20 nuclei, five had $7_{II} + 14_{I_1}$ (fig. 17), six had $8_{II} + 12_{I_1}$ (fig. 16), and five had $1_{III} + 7_{II} + 11_{I_1}$ (fig. 19). One cell had $9_{II} + 10_{I_1}$. Trivalents were found in eight nuclei.



FIGS. 13-21.—Fig. 13, *R. blanda* (7N44), $2n=21$, first metaphase with $5m+2u+21$. Fig. 14, *R. blanda* (7N44), $2n=21$, first anaphase, two univalents dividing at equator, one pair lagging. Fig. 15, *R. macounii*, $2n=21$, diakinesis with $7m$. Figs. 16-18, nuclei of *R. villosa*, $2n=28$, in early diakinesis with $8n+121$, $7n+141$, and $2m+5n+121$ respectively. Fig. 19, *R. villosa*, late diakinesis with $1m+7n+111$. Fig. 20, metaphase complement in *R. villosa* with $2m+6n+101$. Fig. 21, metaphase complement in *R. rubrifolia*, $2n=28$, with $1m+8n+91$.

In the early diakinesis nuclei shown in figures 16, 17, and 18 there are clearly 14 chromosomes with a high chiasma frequency, and 14 with a very low chiasma frequency which may be associated with each other or with the regular pairs by terminal chiasmata only. The contrast between the chiasma frequencies of these two sets of 14 chromosomes is shown graphically in figure 23. In three nuclei at early diakinesis there was a total of 67 chiasmata among the seven normal pairs, and only 22 of them were terminal (table VII). The

TABLE VII
ANALYSIS OF CHIASMATA IN *ROSA VILLOSA*

STAGE	NO. OF NUCLEI	NO. OF PAIRS	TOTAL NO. OF XTA	MEAN NO. OF XTA PER BIVALENT	NO. OF TERMINAL XTA	TERMINAL- IZATION COEFFI- CIENT	HIGHEST NO. OF XTA OBSERVED IN ANY PAIR
Early diakinesis..	3	21	67	3.2	22	0.33	4
Diakinesis							
slide 1.	6	42	110	2.6	46	0.42	4
slide 2.	8	56	111	1.98	58	0.52	3
Metaphase.	3	21	37	1.76	33	0.89	2

mean chiasma frequency of 3.2 per bivalent is the highest yet found at this stage of prophase in a rose. In eight cells in the same locus at late diakinesis, the seven regularly pairing bivalents were again distinguished (fig. 19), and the mean number of chiasmata per bivalent was found to be 1.98. In table VII it is shown that the process of terminalization of chiasmata in *R. villosa* goes on as in other roses (12). As meiosis proceeds, the mean number of chiasmata per bivalent decreases but the proportion of total chiasmata that are terminal increases. At first metaphase terminalization is practically complete (fig. 20), and the terminalization coefficient is 0.89 (table VII). This may be due to a genetic property or to the absence of structural hybridity, as between the normally pairing chromosomes among the modern roses of the Caninae.

A metaphase plate with $1_{III}+8_{II}+9_1$ in *R. rubrifolia* Vill., another member of the Caninae with $2n=28$, is shown in figure 21.

Figures 16, 17, and 18 show that the univalent chromosomes at diakinesis are noticeably less condensed than their paired fellows. It has always been difficult to understand why hybridity should pre-

vent the pairing of one-half of the complement while not affecting the other. On the precocity theory of meiosis, and the theory of the origin of sex chromosomes which follows from it (DARLINGTON 6, 8),



FIG. 22.—Somatic metaphase from root tip of *Rosa* sp., $2n=35$, section Caninae

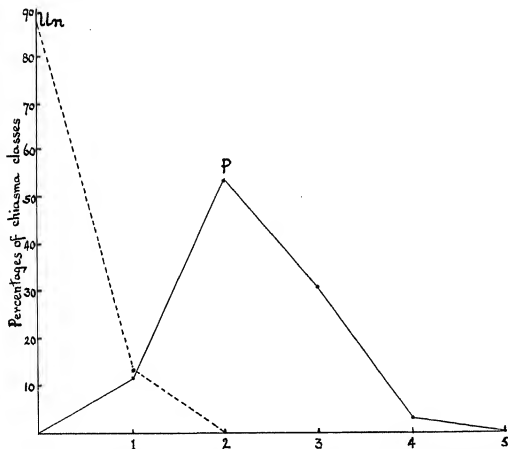


FIG. 23.—Percentage frequency polygons of numbers of chiasmata in bivalents (or their equivalents in unpaired chromosomes and multivalents) in the two sets of chromosomes of *R. villosa*; *p*, paired set, *un*, unpaired set. Data from 14 nuclei at late diakinesis.

this is intelligible; for it may be supposed that the 14 unpaired chromosomes have a genetic property of differential precocity producing the same effect as that in the sex chromosomes in animals.

Thus the possibilities of genetically caused abnormal zygotene pairing are as follows:

1. *Incomplete precocity* of the prophase (for example, *Matthiola*). The prophase is intermediate in precocity between a normal mitotic and normal meiotic condition; this is expressed in long chromosomes at first metaphase, that is, reduced condensation.

2. *Differential precocity*, the two known types of which are: (a) In *sex chromosomes* there is a differential precocity of the chromosomes themselves. The unpaired sex chromosome is itself precocious, as is the prophase, and so restores normal mitotic conditions for itself. This is expressed in precocious condensation and precocious splitting as in ordinary mitosis (DARLINGTON 6). (b) In the *unpaired chromosomes* of the Caninae the prophase contraction begins later than in their paired fellows. They presumably have divided before they condense and therefore cannot pair; a condition that was suspected by HUSKINS (14) to be a cause of asynapsis in dwarf oats and in sorghum.

Somatic chromosomes of Caninae

A somatic metaphase plate from a root tip of a member of the Caninae is shown in figure 22. This plant, still a seedling, was raised from seed collected in Persia by C. D. DARLINGTON. It is a pentaploid with 35 somatic chromosomes. These show both primary and secondary constrictions, as in diploid roses, and resemble the chromosomes of *Oenothera* (DARLINGTON 7, fig. 22).

Summary

1. Multiple pairing of chromosomes due to reduplication and reciprocal translocation of segments has been found in diploid roses belonging to the species complexes *Rosa multiflora*, *R. blanda*, *R. woodsii*, and *R. pisocarpa*. Gametes with eight chromosomes are sometimes produced, but no diploid has been found possessing a reduplicated whole chromosome.

2. A plant of *R. pyrifera* Rydb. (group of *R. woodsii*), previously reported to have $2n=16$, has a small unequal pair which are really reduplicated homologous fragments of approximately half a chromosome. They pair with each other and with two different pairs.

3. Both fragments fail to pair in a proportion of cases. The larger, f' , consists of two segments, homologous with two different pairs. This reduces its pairing frequency (in agreement with the chiasma theory of chromosome pairing) to less than that of the shorter fragment, f . The plant is $4x$ for one short segment and $3x$ for another. This is the only example of fragments yet found in *Rosa*.

4. Multivalent associations of chromosomes are frequent among tetraploid roses, but are relatively rare in hexaploids and octoploids.

5. Triploids with a high proportion of trivalents at diakinesis and first metaphase have been found in diploid cultures of *R. blanda*, *R. macounii* (group of *R. woodsii*), and *R. pisocarpa*. These probably originated from unreduced gametes and arose without hybridization.

6. The unbalanced tetraploid *R. villosa* (section Caninae) shows a high chiasma frequency among 14 chromosomes and a very low frequency among the other 14, which usually occur as univalents. In 75 per cent of the nuclei examined, from one to four of the latter were paired terminally with each other or with one of the regular pairs. Trivalents were found in eight nuclei out of 20. Failure of pairing of the chromosomes is probably due to differential precocity in prophase development, not to hybridization.

7. The chiasma frequency of the fourteen regularly pairing chromosomes in *R. villosa* is 3.2 chiasmata per bivalent at early diakinesis, and terminalization is almost complete at first metaphase.

8. The types of structural change and the different kinds of polyploids found in *Rosa* are described. The exceptional types of pairing found are in conformity with the chiasma theory of pairing and are in three instances such as would be predicted on that theory.

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DIVISION OF THE GENERATIVE NUCLEUS IN THE POLLEN TUBE OF LILIUM

JOSEPH O'MARA

(WITH PLATE IX AND SIX FIGURES)

Most cytological investigations of the higher plants during recent years have been concerned with mitosis in sporophytic generations, particularly behavior at meiosis. The first important investigation dealing with the haploid generation was that of STRASBURGER (3). Later NAWASHIN (2) investigated *Lilium martagon*. WELSFORD (5) studied the formation of sperm nuclei in *L. auratum* and *L. martagon*. TRANSKOWSKY, with material artificially grown on agar-agar, determined the gametophytic development of *Convallaria majalis*, *Galanthus nivalis*, and *Hemerocallis flava*.

MATERIALS AND METHODS.—The figures upon which the present study is based were obtained from pollen tubes of *Lilium regale*. Flowers were pollinated in the morning and worked upon at different times during the following day. Since there is considerable variation in the rate of development of the tubes, all stages were usually found in the preparations of a single day. The material was prepared according to the following technique.

The styles and stigmas were removed from the flowers by breaking the style at the top of the ovary. A longitudinal slit was made through the style, which was then opened up, exposing the pollen tubes. These were removed with a pair of fine forceps and laid on a dry clean slide. The slide was then immersed in the fixative and left for two or more hours. The most satisfactory preparations were obtained when Darlington's modification of the Flemming solution was employed as the fixative. A much sharper stain was obtained by bleaching in a 10 per cent solution of hydrogen peroxide in 80 per cent alcohol than was obtained by the usual bleaching in an aqueous solution of hydrogen peroxide. After the material had been sufficiently bleached, it was stained with crystal-violet-iodine.

All drawings, except figure 4, were made using the 100× fluorite objective, 15× ocular, and a Zeiss camera lucida.

POLLEN-TUBE DEVELOPMENT IN LILIUM

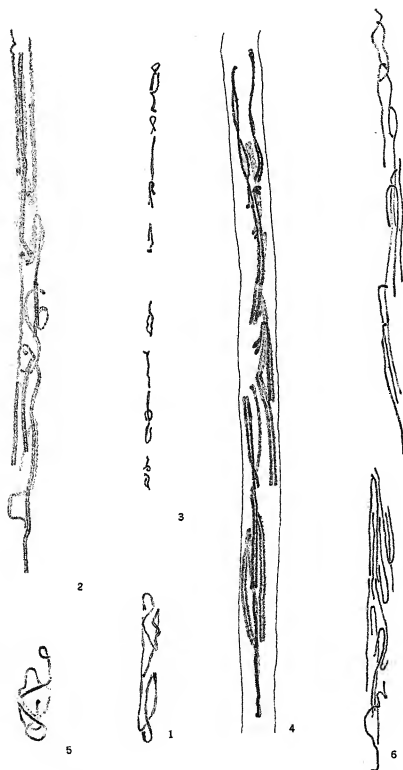
Upon germination of the spore, the characteristic tube appears from the fissure of the ruptured exine and grows rapidly down the hollow interior of the style. The tip of the tube is characterized by a slight swelling and hyaline area. As the tube elongates, the vegetative nucleus usually enters it and passes down, followed at a variable distance by the generative cell, which can be distinguished by its darker cytoplasm and nucleus. Between the two cells can be observed a sharp line of demarcation which is apparently due to the different fixation reactions of the generative and vegetative cells. The generative cell is long, with either pointed or blunt ends, and nearly fills the cross-section of the tube. The generative nucleus is ellipsoid in the beginning and is in an early prophase stage even when it first enters the pollen tube. The vegetative nucleus has no constant shape, however, and seems to change its form as various forces act upon it, that is, forces such as pollen tube contour, cytoplasmic streaming, and generative cell proximity. The vegetative nucleus is variable in behavior as well as in form. It may pass through the tube before, after, or may even accompany the generative cell (TRANSKOVSKY 4). Usually it precedes the gametic cell and rarely follows. When it accompanies this cell, it is constricted up against the pollen tube, so that its length may exceed the total length of the generative cell (fig. 7). The vegetative nucleus has weak staining properties, and shows no structure but that of a fine reticulum of anastomosed chromatin threads. The generative nucleus shows strong basic staining properties, however, and can be sharply stained at the earliest stages. Figure 7 illustrates the relative staining properties of these nuclei. The chromosomes of the generative cell are double from the beginning and are extremely long and convoluted, twisting and winding about through the interior of the nucleus very intricately (fig. 1). As the cell passes down the tube it increases greatly in length, and its nucleus also increases in a like manner, so that often a nucleus will have a diameter of $4\ \mu$ and a length of $90\ \mu$. The nucleus may increase in size to such a degree that it occupies the major portion of the cell. The chromatin threads become more and more loosely arranged and thicker in diameter. This nucleus resembles very remotely that of the characteristic somatic cells, and its chromo-

somes show none of the structural regularity which is so common in root tip mitoses. The threads at prophase are not solid double rods of chromatin which are bent through the interior and around the periphery of the nucleus, but represent thin, long, granular threads which are loosely wound and twisted through the nuclear interior (fig. 1). Although the fixing and staining seemed to be rather sharp, no granular structure, such as has been illustrated by many investigators, could be observed. The successive stages of prophase are passed through, and finally the nuclear membrane disappears and the chromosomes lie in the generative cell cytoplasm (figs. 2-4). At this stage the behavior differs considerably from that usually met with in connection with dividing nuclei.

The chromosomes of the generative nucleus, instead of forming an equatorial plate immediately upon the disappearance of the nuclear wall, remain in their same relative positions in the cytoplasm of the generative cell. The threads at this stage vary greatly in length in different cells, or even in chromosomes of a single cell.

Twists among the threads of an individual chromosome are frequent. Every chromosome, because of its length and involved association in the long nucleus, has usually one twist, and some chromosomes have been observed with as many as six such associations (fig. 8).

The split chromosomes are very long and are often much intertwined, even at this late stage (figs. 2-4, 9, 10, 12, 13), but the twelve pairs can usually be counted easily. This early metaphase stage seems to persist for a comparatively long time, since figures of this stage are common in all preparations. The threads usually lie longitudinally in the tube, and exhibit many interesting features. The paired threads are not straight rods but have more the appearance of wavy lines when viewed from a position where they seem to be single (figs. 2, 12). The pair of threads, not the half-chromosomes individually, follow this wavy course. If the two threads lie parallel and in the same optical plane, they often seem to be paired rows of chromomeres. This appearance is due to the fact that only the crests of the wavy chromosomes are in sharp focus, and the space between the crests forms a hazy bridge. Thus the chromosomes seem to be paired rows of chromomeres. NAWASHIN and TRANSKOWSKY, how-



FIGS. 1-6.—Fig. 1, metaphase; no equatorial plate nor trace of spindle fibers; fig. 2, metaphase; 12 chromosomes in single line in generative cell; fig. 3, metaphase; outline of pollen tube indicated; twists in sister-threads common; fig. 4, telophase; chromosome groups drawn close together in order to have whole figure on plate; ten Vs and two rods present under normal conditions; fig. 5, small portion of sperm nucleus showing solid convoluted threads which appear after reorganization of telophase clump; fig. 6, long, thin, and twisted double threads observable at earliest stages of prophase; chromosome interchange could very easily take place at this stage also. $\times 1550$.

ever, have illustrated chromomeres in *Lilium* and *Galanthus*. The chromosomes seem to vary greatly in length at this stage.

One generative cell had chromosomes $18\ \mu$ in length, and another had chromosomes of $38\ \mu$. This seems to differ from the somatic and meiotic behavior, wherein the chromosomes seem to have a constant length. The somatic chromosomes of this species are approximately $16\ \mu$ in length, and the meiotic, approximately $8\ \mu$ at metaphase. The positions taken by the chromosomes are also variable; some cells contain compact masses of chromosomes which are impossible to differentiate. In rare cases the chromosomes are strung out linearly, so that a line of twelve individual chromosomes passes from one end of the generative cell to the other (fig. 3). As the time for division approaches, however, the chromosomes seem to form a more compact arrangement and to attempt the formation of an equatorial plate. Where the tube is narrow, however, there are obviously more chromosomes than can be accommodated by its small diameter. In these instances the chromosomes form a compact cylindrical mass of chromatin whose individual constituents cannot be identified (fig. 14). Pairs of chromosomes which could not possibly lie on a plate are frequently observed, and it seems to be true that no normal equatorial plate is present. Some tubes, because they are wider at the generative cell, allow for a looser metaphase association, and in these tubes the relationships are much clearer. In these instances the chromosomes orient themselves so that their constriction points are in an approximate plane (fig. 9), although figures where these constrictions can be seen to lie in exactly one plane have never been found. The chromosomes show definite constrictions, but not the slightest evidence of spindle fibers is present. STRASBURGER and HERRIG (1), however, have reported that an achromatic figure is formed in other species of *Lilium*, and that definite spindle fibers of short duration are formed. NAWASHIN and TRANSKOWSKY, working with *Lilium martagon*, *Galanthus nivalis*, and *Convallaria majalis*, found no spindle fibers nor achromatic figures. From his own observations on *Hemerocallis flava*, TRANSKOWSKY has concluded that the absence of achromatic figures and spindle fibers is due to the "Raumverhältnisse" of the generative cell and pollen tube. After the actual division, the chromosomes should pass through the tubes with the constrictions foremost. That they do not do so is interesting.

The meiotic chromosomes of *Lilium regale* have ten approximately median and two terminal or subterminal constrictions. The spindle fibers are usually attached to the chromosomes at these points. In this material, however, no spindle fibers could be observed at any stage of division. Not only were these attachment fibers apparently lacking but no evidence from the chromosomes themselves indicated that they were being "pulled" from the middle of the division figure (figs. 6, 14). No stretched portion of the chromatin was observed at or near the constriction, nor did the figures indicate that any force whatever was acting upon the chromatids. The individual chromosome configurations are very variable and peculiar when compared with somatic chromosomes. Threads are also single at this stage, and show no sign of the double structure which has been observed in somatic chromosomes at anaphase (fig. 6).

After the chromosomes reach opposite ends of the generative cell, they form the characteristic clump which is so common at the telophase of somatic divisions. The generative cell seems to elongate somewhat during this division, and now has a long constriction in the area between the two chromosome groups. The clumps begin to loosen slowly and to assume the more usual form of a resting nucleus. The threads become more and more loosely associated and the nucleus increases in size. The exact behavior of the generative cell at this point is difficult to determine. Often the constriction in its center seems to separate the generative cell into two sperm cells. In a great many cases, even when the sperm nuclei were very far from each other, a thin strand of generative cell plasma could be seen connecting them. Regardless of which process the generative cell undergoes, two naked sperm cells are soon found in the cytoplasm of the vegetative cell. As the chromosomes which clump at telophase begin to loosen and form a new nucleus, the cytoplasm of the generative or sperm cell begins to merge slowly with that of the vegetative cell.

No figures were found which gave any indication as to exactly how this dissolution takes place. That it does so is evidenced by the facts that the outline of the generative cell becomes more and more difficult to follow and that naked sperm nuclei are found in the tubes and at fertilization. The cytoplasm of the vegetative cell appears vacuolate at this stage, especially in the region toward the spore. The two

sperm nuclei lie in the cytoplasm of either the generative, sperm, or vegetative cells, and retain a remarkably constant form considering the forces which may act upon them. They are usually ellipsoid and from three to five times as long as broad. These nuclei have peculiar features also.

No characteristic somatic nuclei are formed as a result of the division of the generative nucleus. Instead of emerging from the telophase clump as a reticulum of finely anastomosed threads, these threads retain the more general features of their structure as chromosomes. The sperm nuclei can be seen to consist of solid, single, individual threads which are more like prophase than telophase chromosomes (figs. 5, 10). The sperm in this species is not to be regarded as a typical somatic nucleus, therefore, but more as a nucleus which resembles closely the interphase stage of meiosis. As the nucleus passes down the tube, it becomes more and more reticulate but never reaches a condition of such complete reticulation as is found in somatic nuclei. An examination of fertilization figures showed that, even at the late stages just preceding fertilization, no typical anastomosed sperm nucleus is ever formed. NAWASHIN has demonstrated that the sperm nuclei of *Lilium martagon* do not pass through a resting stage.

Discussion

GENERATIVE CELL DIVISION

One of the most interesting factors in the division of the gametic cell of *Lilium regale* is the apparent absence of an equatorial plate. In no figure examined was the formation of such a structure observed. Although the variations in chromosome behavior seem to be relatively great, a regular distribution of twelve chromosomes to each pole was observed. In all telophase figures examined, twelve chromosomes could be easily counted at each pole. The haploid number of all plants examined was twelve. These two facts, the regularity of division and the maintenance of a constant specific number, establish definitely the regularity of the generative cell division. This division is perfectly normal, therefore, and is accomplished without the formation of an equatorial plate. In view of these few facts, an equatorial plate would seem to be an unnecessary factor in

the process of mitosis. There are, however, certain other factors involved. Certain configurations in the cells suggest that an attempt was made at the formation of such a plate (fig. 9); but at the same time it was also definitely established that in no case was a recognizable plate formed. However, a diagonally oriented plate would present a condition which would allow for plate formation. No such arrangement was found, and in many cells such a structure was apparently impossible (figs. 4, 13). The remaining explanatory factor seems to be that a plate actually is formed, but the movements of the generative cell, in passing down the tube, disrupt it and scatter the chromosomes. That such a situation is possible but very improbable is indicated by all figures examined. Occasionally the plate could be observed, but at no stage was there a chromosome arrangement explainable by the assumption of such a structure. In view of these facts, the conclusion apparently must be drawn that an equatorial plate is not a necessary and indispensable structure or condition in the process of nuclear division.

Not only is the plate lacking, but at no stage in the complete division could the presence of spindle fibers be demonstrated. Numerous figures were examined for evidence of these threads, and in no case could they be detected. In all the metaphase and telophase figures observed, the chromosomes gave no indication that they were being separated and drawn to the poles by any connecting thread. The chromosomes exhibit, in certain preparations, fiber constriction points both at metaphase and telophase. Since in ordinary cells the spindle fibers seem to be attached to the chromosomes at the constricted points, and these points are usually foremost in passing to the poles, the actual separating force, regardless of its nature, may be assumed to be active (or most active) at these points. That this species has ten median and two terminal or subterminal constrictions has already been established. If the division were a normal one, the chromosomes would orient themselves so that their constriction points would lie on the equatorial plate and would then separate first at these points. This behavior would result in a definite and constant telophase arrangement. The chromosomes which possessed terminal or subterminal attachment points would pass to the poles as straight rods, and those which had median attachment points

would separate into Vs. In this particular species ten Vs and two rods should be present in every telophase set of chromosomes. Furthermore, in normal cells the rods and the arms of the Vs are straight, indicating that they are subject to some tension. Although no equatorial plate is formed in the cells, if the force or mechanism of nuclear division is the same here as it is in all other cells, then the chromosome configurations at telophase would be expected to correspond to the ordinary configurations at this stage. No such constant or expected arrangement was found. All figures showed very irregular chromosomes. The constriction points were foremost in a rather small percentage of the observed cases. In no generative cell was a complete set of chromosomes found with the constricted point preceding the remainder of each chromosome. The chromosomes were not usually straight, but formed rather irregular rods and loops which seemed to be unexplainable on a fiber separation basis. A careful examination of the shapes of the telophase chromosomes showed that they pass to the ends of the generative cell as irregular rods in a great number of cases, instead of separating as Vs (fig. 6). From the appearance of the material alone it was evident that the usual force of chromosome division at mitosis is non-operative in the generative cell. Certain other factors are present at the division of this cell, however, which are found at no other cell division in the life history of the organism. That these factors could influence the mitotic behavior seems probable. STRASBURGER and HERRIG have both reported spindle fibers in the pollen tube. NAWASHIN, however, in view of the odd shapes of the telophase chromosomes and the absence of spindle fibers in his material, has concluded that the chromosomes go to the poles by some type of movement of their own.

The two influences on the chromosome shapes in the generative cell which most probably would be of importance are the streaming of the cytoplasm of the generative cell and the alterations produced in the cell during its movement down the tube. In all ordinary tissue cells which are dividing, the achromatic figure usually forms a clear zone in the cytoplasm. In the generative cell, however, where no achromatic figure is formed, the movement of the cytoplasm might possibly affect the chromosome configurations.

The movement of the generative cell might also be responsible for

the chromosome configurations. In the spore, the shape of this cell is that of a crescent. The pollen tube places definite limitations on the shape of the generative cell, however, and consequently the form of the cell in the spore bears a remote resemblance to its form in the tube. The diameter of the tube is much too small to accommodate the generative cell and the streaming of the vegetative cell. The gametic cell in the pollen tube is long, and small in diameter, therefore, and moves down the tube as a long cylinder. The ability to change shape seems to prove that this cell is plastic and easily influenced by external conditions. If in its movement down the tube it should change shape, as a result of the streaming of the vegetative cell cytoplasm or the contour of the tube, this change would be expected to affect also the chromosome configurations, since, owing to the narrowness of the generative cell, each chromosome lies close to the cell wall and tube wall. As a result of streaming of the cytoplasm of the generative cell and of movement of the generative cell down the tube, therefore, the normal chromosome configurations at telophase could be altered to those which are actually observed.

An examination of the relative positions of the chromosomes at both ends of the generative cell seems to indicate that the time of division is not so precise at this division as it is in ordinary cells (fig. 14). The chromosomes, therefore, neither occupy a position in one plane nor undergo division at precisely the same time, as is common in most cells. These facts seem to demonstrate that the chromosome is more of an individual unit in division than has been assumed. The chromosomes in the generative cell seem, without spindle fibers, equatorial plate, and achromatic figure, to function as autonomous units. This division would seem to invalidate those theories of mitosis which postulate a simultaneous division of chromosomes lying in one plane, resulting from cytoplasmic streaming, balanced electrical and magnetic phenomena, or delicately unstable colloidal systems.

CYTOLOGICAL BASIS FOR INVERSIONS AND TRANSLOCATIONS

The sperm nuclei which result from division of the generative nucleus seem to afford an opportunity for correlating certain genetic and cytological phenomena. These nuclei present unusual structural

relationships which can be observed clearly in late telophase stages. In somatic divisions the chromosomes seem to be arranged regularly at telophase with little or no interlocking. As they pass into the resting stage, the arrangement of the individual chromosomes can no longer be followed. In the sperm nucleus, however, each chromosome maintains its structural identity. The chromosomes may be so twisted that one segment is in contact with another segment of the same chromosome, or non-homologous chromosomes may be interlocked.

Inversions, translocations, segmental interchange, and deficiencies are commonly produced by the effect of X-rays on pollen grains of plants and sperm cells of animals. If the X-rays cause breaks in the chromosome threads which may reunite in new associations, the results may be explained on the basis of the structural relations of the chromosomes as found in the gametic nuclei. If a single chromosome forms a loop, a break at the point of contact might produce an inversion. Segmental interchange would result if two chromosomes were broken at the point of contact, followed by a reunion of non-homologous segments. It is possible, of course, that no such intimate contact of chromosomes is necessary for explaining changes in structural relationships, but the fact that such a physical relation of the chromosomes is found is at least suggestive.

Summary

1. The gametophytic development, after the formation of the mature spore, is described for *Lilium regale*.
2. Explanations for the peculiar configurations of generative cell chromosomes are presented.
3. Evidence is presented that the chromosome is more of an auto-some unit at mitosis than has previously been assumed.
4. A cytological explanation of the mechanism of the formation of chromosome interchanges is presented.

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EXPLANATION OF PLATE IX

FIG. 7.—Metaphase showing the lack of equatorial plate and numerous twists in sister-threads.

FIG. 8.—Metaphase in a wider tube showing, apparently, an attempt at plate formation with constrictions in an approximate plane.

FIG. 9.—Sperm nucleus immediately after the reorganization of the telophase clump. The solid chromosome rods can be seen in place of the usual fine reticulum. This structural relationship is of possible genetic importance.

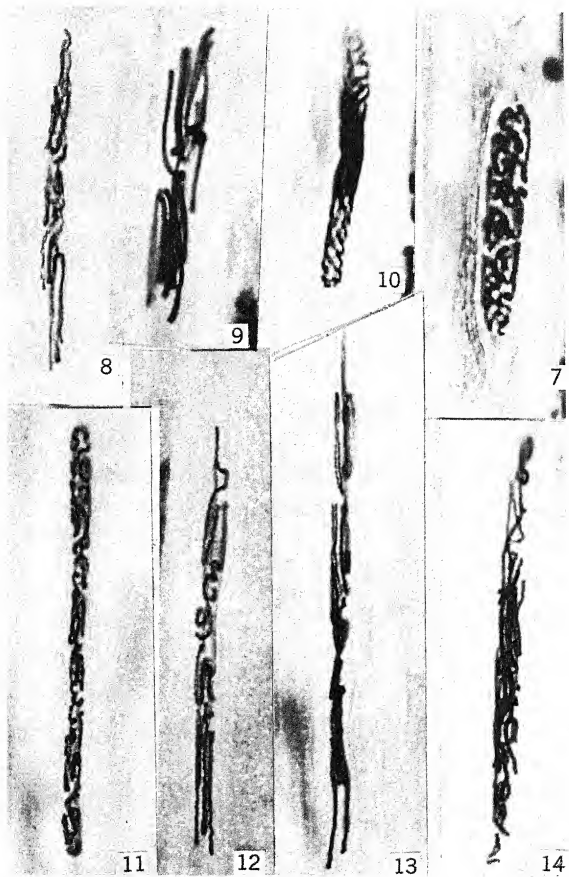
FIG. 10.—Generative nucleus with vegetative nucleus constricted against tube. The relative staining properties of these nuclei are apparent.

FIG. 11.—Usual structure of the generative nucleus at early prophase. The double threads can be seen in many places.

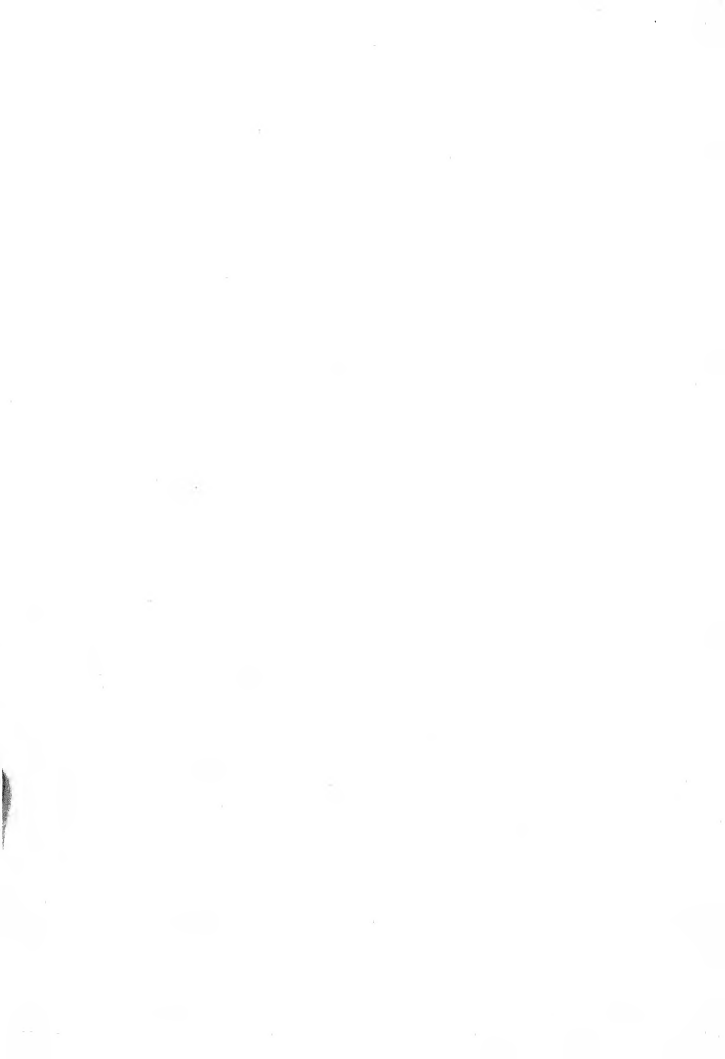
FIG. 12.—Metaphase involving chromosomes of the generative cell.

FIG. 13.—Metaphase of generative cell.

FIG. 14.—Anaphase of generative cell chromosomes; no equatorial plate present; chromosomes at ends of figure have obviously divided much earlier than those in the middle.



O'MARA on LILIUM



DETERMINATE CLEAVAGE POLYEMBRYONY, WITH SPECIAL REFERENCE TO DACRYDIUM

JOHN T. BUCHHOLZ

(WITH TEN FIGURES)

Introduction

A distinction may be made between the two kinds of cleavage polyembryony found in conifers: determinate and indeterminate. In the indeterminate condition there are no indications, from the appearance of the early embryo, that a particular one of the embryonic units has a distinct advantage during embryonic competition. Any one of several embryos derived from the same zygote may become the survivor. In determinate cleavage polyembryony one embryo, usually the terminal one, is more favorably situated than the others, and this is ordinarily the successful embryo. In *Dacrydium*, for example, an embryo unit found at the tip in the group of embryo initials at the end of a prosuspensor is more favorably situated, while one of those in the group immediately behind it can become functional only if the terminal embryo becomes aborted. Here we find an organization in which the position of the various embryonic units, from the earliest stages, usually determines which one is to contribute the successful embryo.

In making comparisons of the various embryogenies of conifers, it has seemed convenient to make this distinction. The description of various embryogenies is greatly facilitated by such a characterization, and the writer has become convinced that the condition of determinate cleavage polyembryony represents one of several steps in the evolution of polyembryony.

A description of the pine embryo has been given (1, 2, 5), and it will not be necessary to redescribe this type of embryogeny in illustrating indeterminate cleavage polyembryony. Any one of the four lower embryo initial cells of the 12-celled proembryo of the pine may contribute the successful embryo of the seed. In the later stages, after the primary suspensors have begun to elongate, or even in the

stages in which the four primary embryos have become completely separated from one another, one may usually recognize, for a time, a moderate degree of equality in the stage of development of the individual embryos belonging to the same system, in the number of cells or length of the suspensor, etc. At any rate, it may be stated that any one of the four embryos has a fair chance of becoming the surviving embryo or "seed germ." Similar conditions are found in *Cedrus* and in *Tsuga* (2, 5).

The embryogeny of *Biota* (4), *Chamaecyparis* (7), or *Cryptomeria* (8) may further illustrate the indeterminate type of cleavage polyembryony. Among these it might be supposed, after considering the early embryo or proembryo, that the embryonic unit which is most nearly terminal in position is the one to be favored during embryonic selection. Among the embryos which result, however, there is usually very little difference in the stage of development, and, for a brief period at least, all or nearly all of them may develop suspensors. It appears that any one of the terminal group of embryos still has a fair chance of becoming the successful embryo of the seed, and these types may be considered as essentially indeterminate.

Whether we consider *Pinus*, *Cedrus*, *Tsuga*, or the embryos of the Cupressaceae,² any of the terminal embryos are favorably situated in comparison with the embryos which may sometimes develop when prosuspensor or rosette cells develop embryos. Thus all embryogenies have an element of being more or less determinate. In fact, a very sharp line of distinction cannot be drawn with certainty between the two types of cleavage polyembryony, but the important thing is that in the indeterminate type there are several embryos situated in the terminal group from which a selection is to be made during later stages of the embryogeny, after suspensors begin to appear on the individual units.

The writer has investigated embryogenies of several genera which appear to fall in the determinate class, notably *Pseudolarix* (5), of which only a few stages have been described. Outstanding examples of determinate cleavage polyembryony are not so well known, however, and it will serve a useful purpose to redescribe the embryogeny

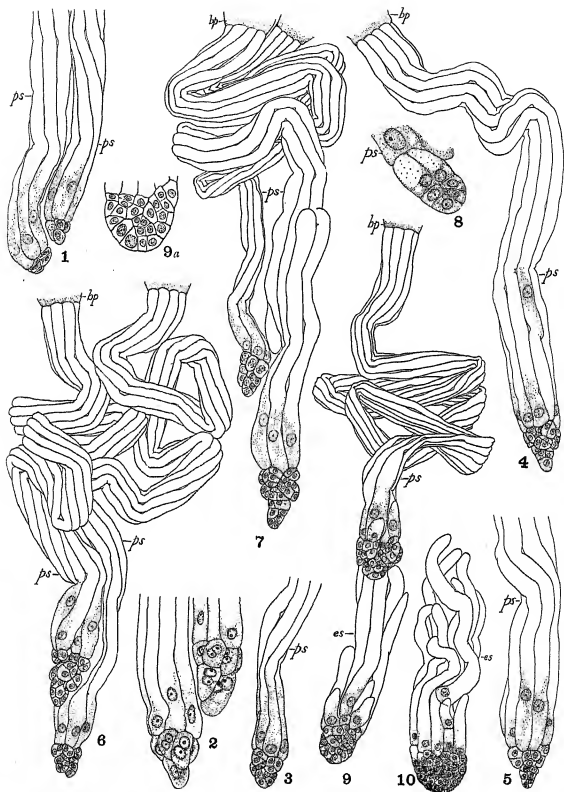
² Where reference is made to families in this paper, a classification (9) which divides the Coniferales into ten families is followed.

of *Dacrydium cupressinum*, including more details and a few stages which are supplementary or additional to the stages previously described (4).

Embryogeny of *Dacrydium*

Figures 1-10 illustrate some of the principal stages in the embryogeny of *Dacrydium cupressinum*. The proembryo is unknown. SINNOTT (11) gives an excellent figure of the archegonium before fertilization and many other details of the morphology. Among his figures he shows one gametophyte containing an embryo which may be in the stage of figures 3-7. Figure 1 shows two embryo systems in one of the earliest stages which was observed. The embryo system at the right shows a typical arrangement of five or six early embryonic cells situated below the prosuspensor. The one at the left bears a similar group of six or seven embryonic cells which have been crushed. The prosuspensor is usually composed of 7-9 cells. In figure 1 the prosuspensors have elongated to a length which is only slightly in excess of the part shown. (The missing upper portion was cut off in dissection.) Each of the five or more binucleate cells at the tip is an embryo initial. The arrangement of these initials varies somewhat, and sometimes a greater number of embryo initials may be found, as many as eight or nine having been observed. Figure 2 shows the lower portion of two adjacent embryo systems in about the same or a slightly earlier stage, under higher magnification. The embryo system at the left has a terminal embryo initial which has been injured and is slightly plasmolyzed. It has a total of six embryo initials, five of which are visible in the drawing. The terminal units are frequently larger than those situated at a higher level, even in this early stage. The embryo system at the right in figure 2 has eight or nine embryo initials. Embryo initials in these early stages are usually binucleate cells.

Figure 3 shows the stage which follows, after the prosuspensor has elongated to one-third or one-half its ultimate length. Each of the six or more binucleate embryo initials is now replaced by a spherical group of cells, and among these the terminal unit is considerably larger than the others. Figures 4 and 5 show similar stages, the former with seven embryonic units and the latter with six. SINNOTT, who gave some attention to the embryogeny of podocarps, un-



FIGS. 1-10.—Stages in early embryogeny of *Dacrydium cupressinum*; explanation in text. *ps*, prosuspensors; *es*, secondary suspensor; *bp*, position of plate or callose plug at upper end of prosuspensor. Figs. 1, 3-7, 9, 10, $\times 75$; figs. 2, 8, 9a, $\times 150$.

doubtedly observed these stages in his sections, for he speaks of the embryo of *Dacrydium* as invariably budding to form additional embryos.

Figure 6 consists of two embryo systems side by side, and shows the characteristic twisting and the great length to which the prosuspensor may stretch; also the manner in which it becomes coiled and twisted. The embryo system below has six distinct embryonic units; the upper one has nine. In the latter (upper system, fig. 6) the terminal unit has become large, composed of nearly a dozen cells; while a few of the units adjacent to the prosuspensor are still in the binucleate embryo-initial stage. It is obvious even in this early stage that this terminal unit will have an advantage in competition with the other embryonic units found within the same system. There is some question, however, as to whether one of the terminal embryos of the lower system (fig. 6), imbedded more deeply within the female gametophyte, will ultimately contribute the successful embryo on account of its more advantageous location, since there are only about half as many cells as in the larger embryo of the upper system.

Figure 7 shows the reverse of figure 6, in having the larger and longer embryo system the one with the greatest number of embryonic units and the shorter one with fewer units. The longer prosuspensor has eight units, the shorter six. All save the terminal embryo units of the upper system are still in the binucleate condition. The prosuspensors in the upper portion are closely coiled and interwoven, and difficult to follow. This is the usual condition of the prosuspensor when several systems are present. The upper portions of the prosuspensors are similarly interwoven but are partially loosened through dissection (fig. 6).

The embryonic units stand out more or less distinctly in all of these early stages, and in each system the terminal embryo is much larger and has more cells than the embryonic units which lie above it. The larger terminal embryo has a distinct advantage over the other units of the same system, and sometimes this may be recognized early, as in the stage shown in figure 2. It is only when this terminal embryo becomes aborted that one of the others has a chance to contribute the embryo of the seed. Thus the determinate nature of the embryo may usually be recognized at a very early stage.

In the stages which follow figures 6 and 7, the largest terminal embryo develops a secondary suspensor by the elongation of embryonal tubes from its proximal end. This massive secondary suspensor thrusts upward the units lying immediately above it, together with the prosuspensor, as shown in figure 9, where three of the four or five embryonic cell groups are shown between the prosuspensor and the secondary suspensor of the terminal embryo. It will be noted that a few of the upper embryos are also beginning to form embryonal tubes. This stage gives the appearance of a suspensor system with embryos between two sections of suspensors.

Figure 10 is a terminal embryo similar to the one in figure 9, in a slightly later stage, the oldest stage which was available. The embryo is still undifferentiated except for the polarity shown by the two ends. It consists of a spherical mass of cells with embryonal tubes elongating from the proximal end to form a massive secondary suspensor, but in this stage there are still no traces of stem tip or cotyledonary primordia.

A large deposit (*bp*) forming a plate or plug, which COKER (10) described as a callose plug in *Podocarpus*, is usually found immediately above the prosuspensor. In some preparations this plug was lost in dissection; in others it was present in the positions which are shaded in the drawings. If rosette cells or rosette embryos are found in other conifers, this plate or plug is found above the rosette group.

No rosette cells were observed. These cells or their remains should be found above the prosuspensor (4). If rosette cells are formed here, it is certain that they are very ephemeral and leave no trace in embryo systems of the stage shown in figure 4.

A careful examination of the embryo of figure 10 gave no indication of an apical initial cell. The embryo of figure 9 still has an apical cell, however, which may be identified at its tip in a deep plane of focus. A view of its optical section in a median focus is shown under greater magnification in figure 9a; figure 9, a surface view of the cells, shows only a small portion of the apical cell. Figure 8 is an embryo of 10-12 cells with its apical cell shown in a median plane of focus still in contact with the prosuspensor. In this case the embryonal tubes are just beginning to elongate.

Nearly all of the individual embryos in the different groups (figs. 3-9) give the appearance of having the apical-cell method of growth.

The apical cell, therefore, appears from the time of wall formation in the various embryo initials, and persists at least until an embryo of the stage of figure 9, comprising approximately 75-80 cells, has developed. It disappears completely before an embryo of twice this size (3-4 times as many cells) has been formed.

Aside from showing the apical cell and determinate cleavage polyembryony, there is still another feature in the embryogeny of *Dacrydium* which is of special interest. There is no primary suspensor; that is, a single elongating cell from each embryo which precedes the formation of embryonal tubes. *Cryptomeria* (8) has no primary suspensor; while *Sciadopitys* (6), *Biota* (4), and *Chamaecyparis* (7), as well as *Pinus* (5), all have primary suspensors, although strictly speaking the last may be considered as having no prosuspensor. In *D. cupressinum* the individual embryo developing at the end of a prosuspensor produces, from the beginning, only a secondary suspensor composed of many embryonal tubes.

Discussion

The embryogeny which has been described for *Dacrydium cupressinum* was previously given in its general outline (4). Very little was stated concerning the apical cell growth of the early embryo, and since not all of the available material had been examined when the scheme of embryogeny was first described, the writer has felt justified, after a more detailed study, in presenting this embryogeny in a more complete form. It may be pointed out that the individual embryos omit the formation of a primary suspensor. This distinction was not made until the embryogenies, and especially the suspensor systems, of *Cryptomeria* (8) and certain Cupressaceae (4, 7) had been carefully investigated and compared. The omission of the primary suspensor need not be taken to suggest affinities between *Dacrydium* and *Cryptomeria*, but should be looked upon as due to parallelism in the evolution of embryogenies within each of two distinct phylogenetic lines. There appear to be other families of conifers among which this distinction may be made. The presence of the primary suspensor (a single-celled elongating cell) may be regarded as a more primitive condition; the omission of this part as a more advanced condition.

The distinction between determinate and indeterminate cleavage

polyembryony may be made for the embryogenies of Pinaceae (Abietineae) (9), where *Pseudolarix* (5) represents the determinate type of cleavage polyembryony, as well as for the Podocarpaceae (9). While the type of embryogeny of *Pseudolarix* has only been hinted at thus far (5), it seems obvious that, on the basis of polyembryony, this type belongs in the same class, or on the same level, as that of *Dacrydium*.

The probable steps in the evolution of polyembryony may be regarded as follows: (a) indeterminate cleavage polyembryony; (b) determinate cleavage polyembryony; (c) simple polyembryony showing definite vestigial traces of determinate cleavage polyembryony; (d) simple polyembryony without traces of determinate cleavage polyembryony. Among the vestigial traces are rosette embryos and rosette cells, or the individual prosuspensor cells which may break away from the group and produce embryonic cells on their tips, as found in both *Cryptomeria* (8) and *Chamaecyparis* (7).

The embryogeny of *Cephalotaxus* (3) is very interesting in the light of these distinctions. There is no apical cell and no primary suspensor. The terminal embryo initials on the end of a prosuspensor all combine to form a single embryo, save for the units represented by the cap cells which become dead cells and break off at an early stage. Above the prosuspensor is a group of rosette cells each of which develops more or less as an independent embryo. In a sense this type may be considered as representing a form of cleavage polyembryony, but as such it is decidedly of the determinate type. The embryogeny would fall into a higher category than that of *Dacrydium*. The latter represents the second (b) category, while *Cephalotaxus* falls more nearly into the (c) category; so much so that the embryogeny has been described as essentially representing simple polyembryony.

Only a few comparisons have been pointed out, but these are probably sufficient to illustrate the value of the distinction between the determinate and indeterminate types of cleavage polyembryony. To include a discussion of the embryogenies of all types which have been observed does not seem necessary at present.

A brief diagrammatic summary of some of the embryonic characteristics, together with the diagnostic taxonomic characters of the families of conifers, is given elsewhere (9).

Summary

1. A distinction may be made between the two types of cleavage polyembryony: indeterminate and determinate.
2. The embryogenies of *Pinus*, *Cedrus*, *Tsuga*, *Cryptomeria*, *Biota*, and *Chamaecyparis* illustrate the indeterminate type of cleavage polyembryony.
3. The embryogeny of *Dacrydium cupressinum* is described in some detail and used to illustrate an embryogeny with determinate cleavage polyembryony.
4. A prosuspensor but no primary suspensors are found in *Dacrydium*.
5. No traces of rosette cells were observed in *Dacrydium*.
6. The early embryo system of *Dacrydium* may be recognized as being compound or composite through binucleate embryo initial cells borne on the prosuspensor.
7. The individual embryos of *Dacrydium* were found to develop by apical cell growth, beginning with the formation of walls in the initial cells and continuing to a stage of more than 75-80 cells; but an apical cell was not found in embryos of three or four times this number of cells.
8. The embryogenies of conifers may be separated into at least four levels with reference to indeterminate and determinate cleavage polyembryony, and simple polyembryony with or without vestigial cleavage polyembryony.
9. These new distinctions in types of cleavage polyembryony or simple polyembryony facilitate the comparison of embryogenies.

The writer is greatly indebted to Professor E. W. SINNOTT for supplying the material of *Dacrydium cupressinum* used in this investigation. The collections were made at Woodlands, New Zealand, in 1911. The younger stages were killed on January 13 and the older stages on January 22.

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NEW OR OTHERWISE NOTEWORTHY
COMPOSITAE. VIII

EARL EDWARD SHERFF

BIDENS PERSONANS Degener & Sherff, Bot. Gaz. 92:205. 1931.—The type of this species was *William Bush* 32, alt. 1200 feet on south slopes in semiarid valley, Palikea, Isl. Oahu, Jul. 7, 1929. It was given its trivial name in allusion to the diverse characters displayed, these suggesting no fewer than three distinct species: *Bidens fulvescens* Sherff, *B. sandwicensis* Less., and *B. conjuncta* Sherff. More recently a further specimen, collected at the same time and place (*Bush* 33), has been sent me by Professor OTTO DEGENER. This has the exact foliar habit of *B. fulvescens* instead of *B. personans*, but resembles the latter in the fact that the immature achenes are but slightly twisted or looped. From my studies upon several cases of hybridity brought to light in the recent collections of Professor DEGENER and his associates, it appears probable that *B. personans* will be found to be a hybrid of *B. fulvescens* with some other species.

Bidens halawana Degener & Sherff, sp. nov.—Planta inferne fruticosa superne herbacea, erecta, glabra, 0.9-3 m. alta, ramis angulatis. Folia petiolata petiolis tenuibus saepius 2-5 cm. longis, petiolo adjecto saepius 7-15 cm. longa, nunc simplicia lamina membranacea ovata moderate grosseve acidentata apice acuta vel subacuminata, nunc 2-3-partita foliolis membranaceis ovatis vel ovato-lanceolatis acriter serratis vel interdum integris lateralibus plus minusve sessilibus. Capitula paniculato-corymbosa, tenuiter pedicellata pedicellis glabris plerumque 1-2 cm. longis, radiata, pansa ad anthesin circ. 6-8 mm. alta et circ. 1-1.6 cm. lata. Involutri bracteae exteriores 4 vel 5 (rarius 3-6) lineari-oblongae, apice subobtusae, glabrae, 1-2.7 mm. longae, quam interiores oblongae vel oblongo-ovatae apice saepe pubescentes plerumque dimidio breviores. Flores ligulati 3-5, flavi, ligula ovato-oblongi vel oblongo-elliptici, apice dentati, 6-8 mm. longi. Paleae demum lineari-oblongae et marginibus hyalinae, concavae, 5-8 mm. longae, in-

volutae et achaenia subtendentes. Achaenia plano-obcompressa, faciebus marginibusque glabra, nigra, exalata, corpore (moderate arcuato vel torto, raro per 1-2 revolutiones voluto) 8-12 mm. longa et 1-1.7 mm. lata, ad apicem vel raro paululum infra apicem biaristata aristis tenuibus parce retrorso-hamosis \mp 1 mm. longis.

Specimens examined: *Otto Degener, Kwan Kee Park, Yoshimasa Nitta, and William Bush (Degener distrib. no.)* 4133, on partly forested lateral ridge, north slope of South Halawa Gulch, about one mile from top of Koolau Range, Isl. Oahu, Hawaiian Isls., Apr. 17, 1932 (type, Herb. Field Mus., 2 sheets; cotypes, Herb. Berl.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.).

Bidens ferax Degener & Sherff, sp. nov.—Fruticosa, erecta, glabra, ramosa, \mp 1 m. alta, ramis tetragonis. Folia opposita, petiolata petiolis tenuibus circ. 2-5 cm. longis, petiolo adjecto circ. 6-13 cm. longa, simplicia vel saepissime tripartita, foliolis lanceolatis vel ovato-lanceolatis, membranaceis vel subcoriaceis, serratis, acuminatis, lateralibus nunc sessilibus nunc tenuiter petiolulatis. Capitula numerosissima, dense corymboso-paniculata pedicellis tenuibus parce glabratis \mp 1 cm. longis, pansa ad anthesin circ. 1.5-1.8 cm. lata et 5-7 mm. alta. Involucri bractee exteriores circ. 4 vel 5, patentes vel reflexae, lineari-oblongae, glabratae vel hispidulae, apice acutae vel obtusae, 1-1.5 mm. longae; interiores oblongae 4-6 mm. longae. Flores ligulati 4-6, flavi, ligula elliptico-oblongi vel cuneato-obovati, apice valde 2- (raro 3-) dentati, 7-9 mm. longae. Achaenia oblongo-linear, valde obcompressa, non vere alata, nigra, unaquaque facierum circ. 4-sulcata, faciebus marginibusque glaberrima, corpore circ. 8-11 mm. longa et circ. 0.5-0.8 mm. lata, supra (vix infra apicem ipsum) sparsim setosa et biaristata aristis tenuibus retrorsum hamosis 0.4-1 mm. longis.

Specimens examined: *Otto Degener, Kwan Kee Park, Yoshimasa Nitta, and Philip Westgate (Degener distrib. no.)* 4117, dry, grassy slope, $\frac{1}{2}$ mile from shore on south side of Keaau Valley, Isl. Oahu, Hawaiian Isls., Mar. 23, 1932 (Herb. Field Mus., 2 type sheets; Herb. Berl.; Herb. Brit. Mus.; Herb. Gray; Herb. Kew; Herb. Mun.).

Bidens aspilioides (Baker) comb. nov.; *Coreopsis aspilioides* Baker, Kew Bull. 1898:153. 1898.—An African species from the region to the south of Lake Nyassa. The type was collected by *Alexander Whyte* and should be at Kew or in the British Museum

of Natural History, but repeated searches in both of these places as well as elsewhere have failed to locate it. The leaves are lanceolate, scabrous, and entire or scarcely dentate. The achenes are glabrous except for the two small, lanceolate pappus awns, and are presumably exalate.

Bidens articulata nom. nov.; *Coreopsis glaucescens* O. & H. in Oliv. Fl. Trop. Afr. 3:389. 1877; *C. abyssinica* f. *latisecta* Vatke, Linnaea 39:499. 1875 (nom. *subnudum*).—Belonging in the section *Steppia*, where it stands closest to *B. rotata* and *B. vatkei*. From *B. rotata* (with achenial bodies 4–6 mm. long and leaves 3-partite) it differs in having achenial bodies 3.5–4.5 mm. long and leaves 3–5-partite, etc. From *B. vatkei* (with achenial bodies 3–4 mm. long, exterior involucre bracts about 6–7 mm. long, leaves slenderly petioled) it differs in having achenial bodies averaging slightly larger, exterior involucre bracts 1.3–1.7 cm. long, leaves subsessile or petiolate with flat, margined petioles, etc.

Bidens acrifolia sp. nov.—Herba forsitana perennis, plus minusve glabrata, caulibus vel ramis gracilibus. Folia opposita, petiolata petiolis usque ad circ. 1 cm. longis, petiolo adjecto principalia saltem 3–4 cm. longa, bipinnatisecta segmentis linearibus firmulis terminaliter subsensim ad apicem acerrimum angustatis, 1–3 mm. latis; summa simplicia lineariaque vel unipinnata. Capitula solitaria tenuiter pedunculata pedunculis \pm 5 cm. longis ramum terminantibus, radiata, pansa ad anthesin \pm 2.5 cm. lata et \pm 8 mm. alta. Involucra plus minusve hispiduli bractee exteriores circ. 12, lineares, acutae, 5–7 mm. longae, quam interiores ovato-oblongae paulo longiores. Flores ligulati \pm 6, flavi, ligula elliptico-oblongi, circ. 1–1.4 cm. longi. Achaenia matura deficientia. Ovaria minima, plana, biaristata aristis tenuibus calvisque.

Specimens examined: *M. P. Dehesa* 1532, alt. 1600 m., Mala Noche, Concordia, State of Sinaloa, Mexico, September (type, Kew; nom. *vulg.* palmita; unico ramo viso).

The general habit, so far as it can be inferred from the single branch examined, is somewhat similar to that of *Bidens geraniifolia* Brandeg. and *B. triplinervia* var. *macrantha* (Wedd.) Sherff. The foliar segments are especially acute, however, and the unbarbed achenial aristae should render distinction easy.

Bidens amplexans Sherff \times *B. waianensis* Sherff, hybr. nov.—*B. waianensi* acheniis supra medium valde recurvatis vel subortis similis, aliter *B. amplexanti* plus minusve similis.

Specimens examined: *Otto Degener* and *D. LeRoy Topping* (*Degener distrib. no.*) 4120, on lantana-covered, somewhat dry slope, along trail leading to top of Keawaula Valley, Isl. Oahu, Hawaiian Isls., Mar. 24, 1932 (Herb. Berl.; Herb. Brit.; Herb. Field Mus.; Herb. Gray; Herb. Kew; Herb. Mo. Bot. Gard.).

Recently Professor OTTO DEGENER forwarded me some specimens collected by himself and Mr. D. LEROY TOPPING along the trail leading to the top of Keawaula Valley, on the Island of Oahu, Hawaiian Islands. One set, numbered by DEGENER 4119, was *B. waianensis*. Specimens are being sent to several herbaria (Herb. Field Mus.; Herb. Gray; Herb. Kew). Another set, numbered by DEGENER 4121, was *B. amplexans*. Specimens of this likewise are being distributed to certain herbaria (Herb. Berl.; Herb. Brit. Mus.; Herb. Field Mus.; Herb. Gray; Herb. Kew). A third set, numbered by DEGENER 4120, was found to be of hybrid material, embodying very emphatically throughout the general habit of *B. amplexans* but approaching *B. waianensis* in the curvature or even the twisting of the distal half of the achenes.

COREOPSIS LEAVENWORTHII curtissii var. nov.—Folia 1-2-pinnata; basalia pinnatim circ. 9-partita, foliolis oblongo-linearibus ad basim sensim angustatis plerumque 2-4 cm. longis et 2-5 mm. latis, lateralibus (praecipue inferioribus) utrinque saepe rursus 1-lobatis lobo parvo linearique; caulina quam internodia nunc multo longiora nunc multo breviora, plerumque 2-pinnata foliolis lateralibus saepius 3 vel 4 jugis, segmentis ultimis omnibus 0.5-2.5 cm. longis et circ. 1-3 mm. latis.

Specimens examined: *Allen Hiram Curtiss* 6734, in dry, pastured ground along the Suwannee River, near Branford, Florida, October 23, 1900 (Herb. Gray, 2 type sheets).

COREOPSIS LEAVENWORTHII lewtonii (Small), comb. nov.; *Coreopsis lewtonii* Small, Bull. Torr. Bot. Club 25:146. 1898.—E specie foliis simplicibus plerumque quam internodiis brevioribus radice saepe perenni differt.

Specimens examined: *Liberty Hyde Bailey* and *Ethel Zoe Bailey* 13047, sands at Sarasota, Florida, Sept. 28, 1929 (Herb. Field Mus.); *Mr. A. H. Curtiss* 1480, swamps and shores, Halifax River, Florida, June (Herb. Berl.; Herb. Field Mus., 2 sheets; Herb. Gray); *Mr. A. A. Eaton* 469, Little River, Dade County, Florida, Dec. 5, 1903 (Herb. Field Mus., 2 sheets); *Albert S. Hitchcock* 139, around flatwood ponds, Myers, Florida, July–August, 1900 (Herb. Field Mus.); *ex herb. S. C. Hood*, low flatwoods, Deep Creek, Florida, June 6, 1910 (Herb. Mo. Bot. Gard.); *Frederick L. Lewton*, Forest City, Florida, 1894 (type, Herb. N.Y. Bot. Gard.); *idem*, Cedar Hammock, Sumter County, Florida, Aug. 3, 1894 (Herb. N.Y. Bot. Gard.); *George V. Nash* 2255, Sanford, Florida, Jul. 24, 1895 (Herb. Berl.; Herb. Field Mus.; Herb. Gray); *Mr. F. Rugel* 133, Florida, 1843 (Herb. Gray; Herb. Mo. Bot. Gard., 2 sheets); *J. K. Small* and *J. J. Carter* 3025, the Everglades, intersecting Long Key, Florida, Jan. 18–26, 1909 (N.Y.); *John Donnell Smith*, pine-barren ponds, Pellicier's Creek, St. John's County, Florida, Mar. 4, 1882 (Herb. Univ. Chi.; Herb. Field Mus., 2 sheets; Herb. Gray); *Mr. S. M. Tracy* 7256, Long Key, Florida, May 8, 1901 (Herb. Berl.; Herb. Field Mus.; Herb. Gray); *idem* 7355, Perico Isl., Dec. 6, 1901 (Herb. Gray; Herb. Mo. Bot. Gard.).

An examination of the foregoing specimens in comparison with a great number of specimens of *C. leavenworthii* proper fails to show more than mere varietal differences. Upon reducing *C. lewtonii* to varietal status under *C. leavenworthii*, we have a total of three varieties for that species: var. *garberi* A. Gray, var. *curtissii*, and var. *lewtonii*. These may be distinguished by the following key:

Folia simplicia var. *δ. lewtonii*

Folia principalia 1–2-pinnata

Foliorum caulinorum foliola terminalia nunc obovata nunc lanceolato-oblonga, 0.8–1.5 cm. lata var. *β. garberi*

Folia caulinorum foliola terminalia (vel eorum segmenta ultima)

circ. 1–3 mm. lata

Folia basalia pinnatim circ. 9-partita var. *γ. curtissii*

Folia basalia integra *C. leavenworthii* sensu stricto

COREOPSIS GRANDIFLORA var. *harveyana* (A. Gray), comb. nov.; *Coreopsis harveyana* A. Gray Synopt. Fl. N. Amer. 1st: 292. 1884.—Foliorum segmentis anguste linearibus vel quidem filiformibus, acheniis paulo minoribus circ. 2 mm. longis differt.

Specimens examined: *Anon.*, Arkansas National Forest, Arkansas, 1909 (Herb. U.S.); *Mr. B. F. Bush* 158, Sheffield, Missouri, June 12, 1894 (Herb.

Berl.); *Frederick V. Coville* 127 Ark., Mountain Park near Little Rock, Arkansas, Jul. 17, 1887 (Herb. U.S.); *Mr. H. Eggerl*, Williamsville, Missouri, June 11, 1893 (Herb. Field Mus.); *Prof. Francis LeRoy Harvey*, cliffs near Fort Smith, Arkansas, June (Herb. Gray, type, cum *C. tinctoria* Nutt. pro parte parva commixta); *Mr. E. J. Palmer* 8120, wet, open hillsides, Malvern, Arkansas, June 23, 1915 (Herb. Mo. Bot. Gard.); *H. S. Reynolds*, Judsonia, Arkansas, May 31, 1877 (Herb. Field Mus.); *Huron H. Smith* 1150, railway tracks, Des Arc, Iron County, Missouri, June 8, 1908 (Herb. Field Mus.).

The type sheet of *C. harveyana* A. Gray bears three specimens. The first at the left is *C. tinctoria* Nutt., having the oblong wingless achenes, the bicolored ligules, the subtruncate style-branch tips, the linear-oblong paleae, etc. of that species. The other two are very distinct, having orbiculate and winged achenes, caudate-conical tips to the style branches, upwardly elongate-attenuate paleae, etc. GRAY clearly was misled by the admixture of the small *C. tinctoria* specimen, for in his Synoptical Flora he separated *C. harveyana* partly on the basis of the "rays sometimes brown-purple at base." Had the rays of *C. harveyana* really possessed the tendency toward a bicolored state, a separation of the type as a new species would have been justified. Since the rays were really yellow, however, the type had nothing left to separate it from *C. grandiflora* proper except the narrower leaf divisions and the slightly smaller achenes. These differences GRAY himself elsewhere (e.g., *C. drummondii* Torr. & Gr. et var. *wrightii* A. Gray Synopt. Fl. N. Amer. 11:291. 1884) considered as connoting a variety.¹

COREOPSIS TINCTORIA imminuta var. nov.—Capitula pansa ad anthesin 1-1.5 cm. lata, bracteis exterioribus \mp 1 mm. interioribus circ. 5-6 mm. longis, acheniis 0.9-1.4 mm. longis.

Specimens examined: *John Michael Holzinger*, Santa Fe, New Mexico, August 13, 1911 (type, Herb. Mo. Bot. Gard.).

Coreopsis killipii sp. nov.—Fruticosa, glabra, 6-12 dm. alta, ramosa ramis tenuibus angulatis internodiis quam foliis longioribus. Folia petiolata petiolis moderate ciliatis circ. 4-13 mm. longis basi in poculum \mp 1.5 mm. altum connatis, petiolo adjecto circ. 1.5-2.5 cm. longa, pinnatim vel subbipinnatim divisa, segmentis ultimis saepius oblongo-linearibus crassiusculis eciliatis planis vel vix revolu-

¹ A supposedly duplicate sheet of the *Harvey* material at St. Louis (Herb. Mo. Bot. Gard.) bears all *C. tinctoria* Nutt.

tis apice acutis subacutisve. Capitula pauca, in pedunculis ramos elongatos superne nudatos terminantibus $\mp 1-1.2$ cm. longis disposita, radiata, pansa ad anthesin $\mp 2.6-2.8$ cm. lata et $\mp 0.8-1$ cm. alta. Involucrum tantum ad basim ipsam hispidum, bracteis exterioribus circ. 8, oblongis, eciliatis, apice obtusis vel rotundatis, 3-5 mm. longis et 1-1.7 mm. latis; interioribus ovato-oblongis, siccis plus minusve atris, apicem versus vix ciliatis, 7-10 mm. longis. Flores ligulati forsitan 5 vel 6, brunneo-flavi, ligula elliptico-oblongi, apice integri vel parce denticulati, circ. 1.2 cm. longi. Paleae spatulato-oblancoolatae, dorso (praecipue ad costam medianam) hispidae ventre glaberrimae, apicem acutum versus ciliatae, circ. 5-6 mm. longae ad corpus achaenii strictim applicatae. Flores tubulosi subbrunneo-flavi, stylorum ramis incrassatis apice subito breviterque conico-appendiculatis. Achaenia valde obcompressa, cuneato-oblancoolata, nigra vel fere purpurascens-nigra, dorso glaberrima, ventre marginibusque perspicue erecto-pilosa pilis elongatis sericeis, corpore 4-5 mm. longa, apice biaristata aristis linearibus stramineis antrorsum hispidis 1.5-2.5 mm. longis.

Specimens examined: *Ellsworth P. Killip* and *Albert C. Smith* 21823, straggling shrub 2-4 ft. high, open hillside, alt. 3000-3200 m., Tarma, Department of Junín, Peru, Apr. 20-22, 1929 (type, Herb. Field Mus.).

The South American species of *Coreopsis* are shrubby and fall into the section *Pseudo-Agarista* A. Gray. Some (e.g., *C. pickeringii* A. Gray) have comparatively large heads and light yellow rays, others (e.g., *C. macbridei* Sherff) have comparatively small heads and rays which at least in the dried state are a brownish yellow. In these latter the involucre at least of the dried heads tend more conspicuously to be blackish. One such species is *C. killipii*. It is perhaps most closely related to *C. macbridei* Sherff and *C. notha* Blake & Sherff. From the former it differs at once in having smaller leaves, fewer and much larger heads, also comparatively (and actually) much larger exterior involucre bracts, etc. From the latter it differs in having smaller leaves, linear-oblong (not lanceolate to oblong) leaf segments, about 8 (not 6 exterior involucre bracts, these 3-5 (not 2-3) mm. long, etc.

Coreopsis nodosa sp. nov.—Fruticosa, forsitan 6-8 dm. alta, ramosissima ramulis nunc subteretibus nunc valde angulatis glandu-

loso-puberulis internodiis numerosissimis plerumque 1-4 rarius usque ad ∓ 10 mm. longis et circ. 1-1.5 mm. crassis. Folia numerosissima opposita petiolata petiolis plus minusve hispidociliatis basi in poculum minutum connatis 1-5 mm. longis (parte inferiore ad ramulos juncta manente post casum foliorum), petiolo adjecto tantum circ. 7-9.5 mm. longa, glabra, pinnatim 3-7-partita segmentis crassiusculis oblongis vel ovato-oblongis sparsim hispidulis apice subobtusum mucronatis plerumque tantum 2-3.5 mm. longis et 0.4-0.7 mm. latis. Capitula tenuiter pedunculata pedunculis (supra summa folia) circ. 1 cm. longis, radiata, pansa ad anthesin ∓ 1.8 cm. lata et 7-8 mm. alta. Involucri basaliter tomentuloso-hispidi bracteae exteriores circ. 8, cuneato-oblongae, longitudinaliter 3-vittatae, glabratae, apice obtusissimae vel rotundatae, 2-3 mm. longae; interiores cuneato-oblongae apice obtusissimae 4-7 mm. longae. Flores ligulati 6-8, claro-flavi, ligula elliptico-oblongi, apice integri ∓ 8 mm. longi. Paleae lineari-oblongae, dorso marginibusque erecto-hispidae ventre glabratae, apice acutae, 5-6 mm. longae, ad corpus achenii strictim applicatae. Achaenia valde obcompressa, cuneate lineari-oblonga, atra, duabus faciebus praesertim ventrali ac marginibus valde erecto-setosa setis longis albidis sericeis, corpore circ. 4 mm. longa, apice biaristata aristis tenuibus stramineis antrorsum setosis 1-1.8 mm. longis, inter aristas poculo minuto coronata.

Specimens examined: *Francis W. Pennell* 13646, open, rocky slope, alt. 2900-3100 m., Ollantaitambo, Department of Cuzco (Cusco), Peru, Apr. 26, 1925 (type, Herb. Field Mus.).

COREOPSIS DELPHINIFOLIA chlooidea var. nov.—E specie foliorum segmentis magna ex parte tantum 0.3-2 (nonnullis usque ad 3.2) mm. latis differt.

Specimens examined: *Samuel Bottsford Buckley*, in pine woods, mountains of Alabama (type, Herb. Gray); *Roland M. Harper* 545, dry pine barrens, Sumter County, Georgia, Aug. 31, 1900 (Herb. Field Mus.).

The plants examined are seen from their caudate-tipped style branches and other characteristic parts to be unmistakably nearest to *C. delphinifolia* Lam. From that species they differ, however, in having the leaf segments narrower by half. It is interesting to note that in the closely related *C. major* Walt. two varieties are dis-

tinguished by their comparably narrower leaf segments, var. *linearis* Small and var. *rigida* (Nutt.) F. E. Boynt.

Coreopsis corninsularis sp. nov.—Herba perennis, erecta, gracilior, caulibus tenuibus angulatis glabris 2 vel 3 ex unico basi circ. 3-4 dm. altis. Folia opposita, plus minusve petiolata petiolis tenuibus usque ad 3 cm. longis basi sparsim hispido-ciliatis, petiolo adjecto plerumque 3-8 cm. longa et 1-6 mm. lata, saepius integra anguste vel late spathulato-linearia crassiuscula faciebus glabra marginibus ciliata apice subacuta vel subobtusa, interdum 3-5-partita foliolis lateralibus multo minoribus et tantum 1-2 mm. latis. Capitula solitaria longe pedunculata pedunculis tenuibus glabris 1-1.8 dm. longis, radiata, pansa ad anthesin 3-5 cm. lata et 10-12 mm. alta. Involucri glabri bracteae exteriores 8-10, lanceolatae vel oblongo-lineares, 3-5 mm. longae; interiores ovato-oblongae, demum circ. 1 cm. longae. Flores ligulati circ. 8, flavi, ligula oblongo-oblanco-lati, apice saepius 3-lobati lobo mediano multo majore apice rursus in 2 dentes inciso, 1.5-2.5 cm. longi. Paleae tenuiter lineares, superne filiformes, 3-4 mm. longae. Flores tubulosi flavi, styli ramis terminaliter caudato-appendiculatis. Achaenia valde obcompressa, dorso convexa, nigra, corpore ipso oblongo 2.5-3.2 mm. longa et (alis exclusis) 1.2-1.5 mm. lata, faciebus glabra vel glabrata, marginibus alata alis expansis membranaceis quam corpore saltem 0.5-0.75 angustioribus, apice papposa 2 fimbriatis squamellis circ. 0.1-0.2 mm. longis.

Specimens examined: *Samuel Mills Tracy* 8542, Horn Island, Mississippi, May 25, 1903 (type, Herb. Mo. Bot. Gard.; cotype, Herb. Field Mus.).

This is an apparently endemic species from a small island in the Gulf of Mexico. It is close in technical characters to *Coreopsis lanceolata* L., from which it differs in its smaller and more delicate leaves, also its smaller external involucral bracts. The habit is strongly suggestive of *C. debilis* Sherff, found on the mainland proper of the southeastern United States, but the fewer and much larger heads, the much longer peduncles, the larger achenes, etc. all separate it from that species.

VASCULAR ANATOMY OF THE TRANSITION REGION OF CERTAIN SOLANACEOUS PLANTS

ALBERT F. THIEL

(WITH NINE FIGURES)

Introduction

In a recent paper on the anatomy of the primary axis of the egg-plant, *Solanum melongena*, the writer (3) reported the results of his studies on root-stem transition. Since the method of transition in the egg-plant differed from that of the potato, as reported by ARTSCHWAGER (1), additional studies were made on six other species of solanaceous plants, including the potato. It was the purpose of this investigation to determine whether or not variations in the method of root-stem transition are common among species of the Solanaceae.

ARTSCHWAGER (1) was the first to give a detailed description of the root-stem transition in the potato plant. He states: "In the change from the exarch to the endarch condition it is noticed first, that the two protoxylem groups of a diarch root begin to swing outward, one following a left, the other a right curve" (fig. 1). At a point just below the cotyledons he found that the primary xylem groups, instead of forming a radial row, come to lie in a tangential plane. The change from the exarch to the endarch condition was completed in the region above the cotyledons.

KING (2), studying *Lycopersicum esculentum*, found that the primary xylem plate bifurcated and differentiated into two distinct bundles. The metaxylem differentiated tangentially toward the periphery of the stele at successively higher levels. The protoxylem maintained its original position until the level just below the cotyledonary plate was reached. Centripetal differentiation of the protoxylem began at this point and continued until the primary xylem groups were almost endarch at the cotyledonary plate.

THIEL (3), in studying the anatomy of the primary axis of the egg-plant, found that the method of transition agrees with that of KING for the tomato, but disagrees with the results of ARTSCHWAGER

for the potato plant. KING did not investigate the changes occurring in the petiole or midrib of the cotyledon. The first change in transition in the egg-plant was a breaking up of the diarch xylem plate and the two primary phloem groups, forming two units of the primary xylem and phloem. At a higher level there is a bifurcation of the metaxylem and an inward differentiation of two of the phloem groups. Near the cotyledonary plate there is a separation of the two primary

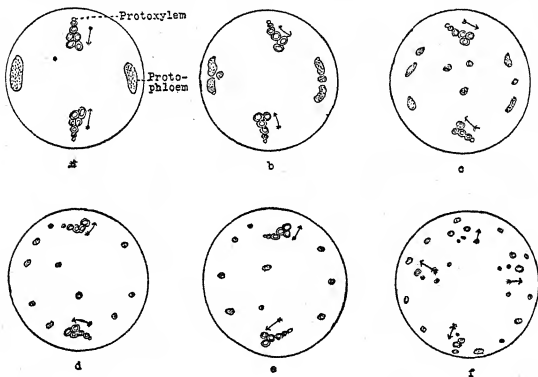


FIG. 1.—Photograph of ARTSCHWAGER's (1) diagrams illustrating root-stem transition in the potato plant; reproduced by permission of M. C. MERRILL.

xylem units formed by the breaking of the original diarch xylem plate. One of these units becomes the vascular trace of one of the cotyledons, and the second unit, that of the other. By inward differentiation and further division, several small primary phloem groups finally come to lie opposite the inner faces of the primary xylem elements. The four remaining groups lying nearest to the metaxylem are gradually inclined in a tangential direction, eventually lying on the outside of the original position of the protoxylem points. This development continues until the bicollateral condition is established. The endarch condition is not attained in the hypocotyl but in the midrib of the cotyledon. The behavior of the primary phloem in the

egg-plant agrees in general with the findings of ARTSCHWAGER for the potato and with KING for the tomato.

MATERIAL AND METHODS.—The solanaceous plants used in this study were: potato, *Solanum tuberosum*; tomato, *Lycopersicum esculentum*; Jimson weed, *Datura tatula*; ground cherry, *Physalis virginiana*; *Petunia acuminata*; and Jerusalem cherry, *Solanum pseudo-capsicum*.

Seedlings, varying in age from 5 to 15 days, were used for each species investigated. Studies were made with plants in which the primary tissues were just beginning to differentiate from the procambium to fully matured primary permanent tissues.

Flemming's weaker killing solution was used, and the usual laboratory methods of dehydrating and imbedding in paraffin were followed. The sections were stained with safranin and light green.

Root-stem transition

The primary root of the potato and the other five solanaceous plants investigated is a diarch, radial protosteles (fig. 2). The method of transition was found to be exactly the same in each of them, and is the same type as that previously reported for the egg-plant. Since the writer's findings for the potato do not agree with those of ARTSCHWAGER, illustrations of the potato will be used in this paper, representing the typical method of transition for each plant studied.

The first change in the transition from the diarch, radial protostele of the root to the bicollateral type of bundles in the cotyledons and stem is a breaking up of the diarch xylem plate and a division of the two primary phloem groups each into three distinct groups (figs. 2, 3). This is followed by a bifurcation of the metaxylem of the two primary xylem units (fig. 4). ARTSCHWAGER's illustrations show that the two primary xylem units do not bifurcate but swing outward, one following a left and the other a right curve (fig. 1). No such condition was observed in the serial sections of many seedlings of different ages studied.

The central group of phloem cells at each side differentiates toward the center of the axis and becomes the inner phloem (figs. 3, 4). Each of the four remaining groups of the primary phloem is gradually inclined in a tangential direction toward the position of the

protoxylem points (figs. 3-5). Just below the cotyledonary plate there is a gradual separation of the two primary xylem units formed by the breaking of the original diarch xylem plate (fig. 5). One of these units, consisting of one protoxylem point, its metaxylem, and the internal and outer phloem groups, becomes the vascular trace of one of the cotyledons; and the second unit, that of the other (fig. 6). THOMAS (4) applies the term "double bundle" to these individual primary xylem units. Their double characteristic is very conspicuous in the ground cherry (fig. 6), while it is not so noticeable in the potato (fig. 7). Near the cotyledonary plate there is a beginning of the centripetal differentiation of the protoxylem and a centrifugal differentiation of the metaxylem.

The behavior of the vascular elements beyond the cotyledonary plate was followed in the petiole and midrib of one of the cotyledons.¹ In the cotyledonary petiole and midrib the protoxylem differentiates adaxially and finally is nearer to the upper epidermis than the metaxylem (figs. 7-9). Simultaneously the metaxylem differentiates abaxially until the endarch condition is fully established (figs. 7-9).

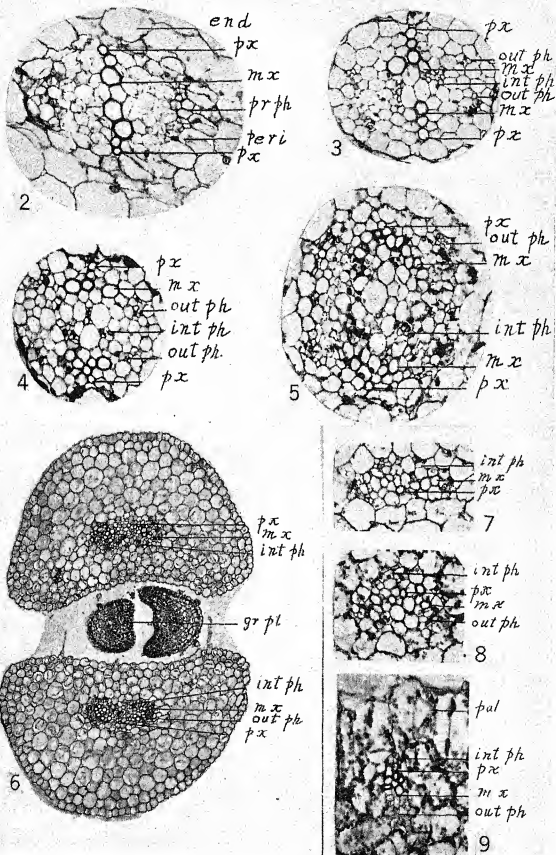
The internal phloem groups are numerous in the cotyledonary petioles (fig. 7). They gradually decrease until there are none remaining in the smaller bundles. In the small bundle shown in figure 9, one small group of inner phloem cells is still noticeable. The outer phloem groups differentiate toward the position originally occupied by the protoxylem point and incline progressively nearer one another until there is complete union of the two groups, thus establishing the bicollateral condition (figs. 6-8).

In the first internode the vascular bundles are completely endarch. Their elements differentiate against those of the hypocotyl slightly below the cotyledonary plate.

Summary

1. Root-stem transition was investigated in *Solanum tuberosum*, *Lycopersicum esculentum*, *Datura latula*, *Physalis virginiana*, *Petunia acuminata*, and *Solanum pseudo-capsicum*.

¹ The illustrations are oriented so that the adaxial surface of the petiole and lamina are toward the top of the page. See, for example, the position of the vascular elements in the lower cotyledon in figure 6. These will be followed until the endarch condition is established.



FIGS. 2-9.—On opposite page

2. The method of transition in each of the plants investigated is the same and agrees with the method in *Solanum melongena*.

3. Illustrations of transition of the potato were used as representative of the other members of the group. The first change from the diarch, radial protostele of the root is a breaking up of the xylem plate and a division of each of the two primary phloem groups into three distinct parts.

4. This is followed by a bifurcation of the metaxylem of the two primary xylem units. These units were not observed to swing outward, one following a left and the other a right curve, as reported by ARTSCHWAGER.

5. The central groups of phloem cells from each side differentiate toward the center of the axis and become the internal phloem. Each of the four remaining groups is gradually inclined in a tangential direction toward the original position of the protoxylem points. This development continues until the bicollateral condition is established.

6. One of the primary xylem units, formed by the breaking of the original diarch xylem plate and consisting of one protoxylem point, its metaxylem, and internal and outer phloem groups, becomes the vascular trace of one cotyledon; the second unit, that of the other.

7. In the cotyledonary petiole and midrib the protoxylem differentiates adaxially and finally is nearer to the upper epidermis than is the metaxylem. Simultaneously the metaxylem differentiates abaxially until the endarch condition is established.

FIGS. 2-9.*—Fig. 2, transverse section of root of potato, showing the diarch, radial protostele; fig. 3, transverse section of hypocotyl showing the two primary xylem units and inward differentiation of two phloem groups; fig. 4, same at higher level, showing bifurcation of metaxylem, the four groups of outer phloem, and several groups of internal phloem; fig. 5, transverse section of hypocotyl 2 mm. below cotyledonary plate showing further bifurcation of metaxylem and the two primary xylem units, each becoming a cotyledonary trace; figs. 2-5, $\times 500$. Fig. 6, transverse section through petioles of cotyledons of the ground cherry, showing position of vascular elements in cotyledons, centripetal differentiation of protoxylem, and centrifugal differentiation of metaxylem. Same situation obtains at this level in the potato plant. $\times 150$. Figs. 7-9, transverse sections through petiole and lamina of cotyledon at successively higher levels; protoxylem differentiates adaxially and metaxylem abaxially until endarch condition is established (adaxial surface of petiole and lamina toward top of page). $\times 500$.

* Abbreviations for all figures: *end*, endodermis; *epi*, epidermis; *gr. pt*, growing point of stem axis; *int.ph*, internal phloem; *mx*, metaxylem; *out.ph*, outer phloem; *peri*, pericycle; *pr.ph*, primary phloem; *px*, protoxylem; *pal*, palisade.

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A NEOGENE SPECIES OF SEQUOIA FROM JAPAN¹

SEIDO ENDO

(WITH THIRTEEN FIGURES)

Sequoia sempervirens Endlicher **fossilis** Endo.—Cone rather small, about 17 mm. in transverse diameter. Cone scales about 6 in one cycle; distal end of the ovule bearing rhomboidal scale 10 mm. wide and 4 mm. high, with surface transversely grooved and radially wrinkled. Peduncle stout, $3 \pm$ mm. in diameter. Leaves with decurrent base, sessile, spirally arranged, of two kinds: (1) those on the lateral branchlets, arranged in two lines, having twisted base, linear or lance-shaped, and bluntly mucronate at apex, 10 mm. long, about 2 mm. wide, upper surface slightly furrowed along the midrib; (2) those on the leading and cone-bearing shoots, arranged in several ranks, rather short, not longer than 5 mm., oblong, curved like a horn and pointed (figs. 1-9).

The distichous foliage does not continue uninterruptedly for any considerable distance, but frequently alternates with the imbricated type. Stomata arranged longitudinally and discontinuously on either side of the midrib on the under surface of the leaves; each stoma ringed by four or five narrow subsidiary cells.

LOCALITIES.—1. Middle Umoregi group; a river bed of Hirosegawa just under the Otamaya-bashi, Komega-fukuro, Sendai; figs. 1, 3, 4, 6 (collected by *S. Endo*).

2. Lower Umoregi group; Sanjunin-machi, Sendai (collected by *S. Endo*).

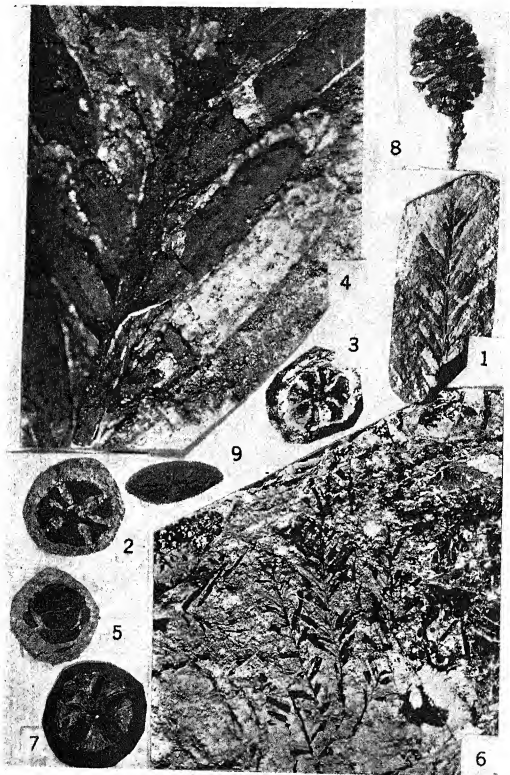
3. Saboyama group; Shiogama-machi near Sendai; figs. 2, 5 (collected by *H. Ozaki*).

4. Sekiya group; Hokine-mura, Shioya-gori, province of Shimotsuke (collected by *H. Niino*).

5. Unknown horizon; Tateyama-shita, Iwayado-machi, Esashigori, province of Rikutiu (collected by *H. Yabe* and *F. Saito*).

The present material is identical in the outer features of the cones

¹ Contribution from the Institute of Geology and Palaeontology, Tohoku Imperial University; Sendai.



FIGS. 1-9.—Fossil and living material of *Sequoia sempervirens* Endlicher: figs. 1, 6, examples of foliage; figs. 2, 3, 5, 7, 8, cones; fig. 4, portion enlarged from fig. 1 to show mode of attachment of leaflets to branch; figs. 2, 3, 7, cross-sections of cones showing characteristic cone scales; fig. 5, ornamentation of escutcheons; fig. 9, surface of escutcheon of fig. 5; figs. 7, 8, living material. $\times 2$.

and leaves with *Sequoia sempervirens* Endlicher, found in the western coastal region of the United States in Oregon and northern California (figs. 7, 8). In the fossils, the distichous foliage does not continue uninterruptedly for any considerable distance, but is in frequent alternation with the imbricated type, as already stated. In this respect also it is closely allied to the existing *S. sempervirens*.

The leaves are not so close to the branchlet as in *Sequoia chinensis* (Endo) (1) and *S. disticha* (Heer) (4), and the cones are conspicuously different from *S. chinensis*.

Sequoia tournaalii (Brongniart) (4), described by A. G. NATHORST from Shimoshino-kinai-mura, Senboku-gori, province of Ugo, is another closely allied species, although not definitely referable to the present species without cones.

The cones and the leaves just described are found with erect stumps, which are covered by a pumice bed, the so-called "Hirosegawa pumice bed," which is about 4 m. in thickness at this place. It is probable that the forest which produced the present erect stumps was destroyed by this volcanic material. In a basal portion of the volcanic materials of this pumice bed there is a plant-bearing deposit, about 3 cm. thick, with abundant leaves and cones of *Sequoia*. Such being the case, it would seem that these *Sequoia* remains had been detached from the present erect stumps.

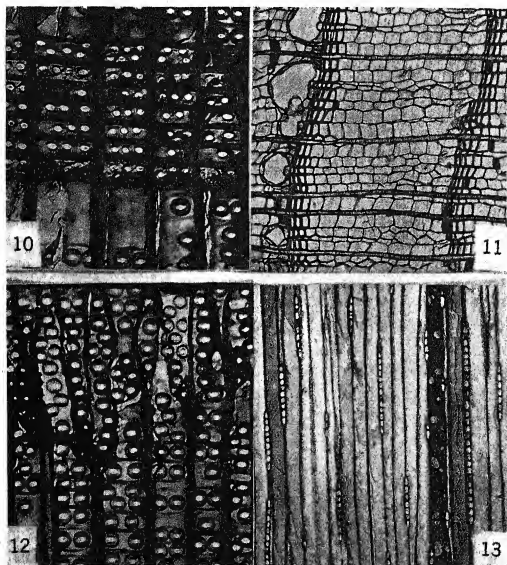
The internal structure of the erect stump was referred by the writer, some years ago, to *Taxodioxydon sequoianum* (Merckl.) Schmalh. erw. Goth. em. (figs. 10-13). This species is similar to *Sequoia sempervirens* in the internal structure, as GOTHAN (2) has stated. Recently TAKAMATSU (6) studied critically the woody structure of the erect stumps at this locality and came to the same conclusion.

GEOLOGICAL AGE.—The three groups cited above from the province of Rikuzen, the Middle Umorigi, the Lower Umorigi, and the Saboyama, are almost certainly Miocene in age, as will be discussed later.

According to SHIMIZU (10) the Cenozoic deposits of the environs of Sendai are divided into groups as follows:

1. Aobayama (Pleistocene); unconformable to the underlying Neogene formations.

2. Upper Umoregi, 10-15 meters; unconformably overlies the next older group at places.
3. Middle Umoregi, 18.5-22.5 m.; unconformable to the underlying group.



FIGS. 10-13.—*Taxodioxylon sequoianum* Gothan, from Middle Umoregi group; a river bed of Hirose-gawa just under the Otamaya-bashi, Komega-fukuro, Sendai: figs. 10, 12, radial longitudinal section of wood; fig. 11, cross-section of wood; $\times 100$; fig. 10, radial longitudinal section of wood showing pits of tracheids; $\times 350$; fig. 13, tangential longitudinal section. $\times 50$.

4. Tatsunokuchi, 30 m.
5. Lower Umoregi, 15.8 m.; this and the upper part of the next or Mitaki group are contemporaneous, although in two different facies of sediments.

6. Mitaki, 20-25 m.; this group shows an apparent gradual transition downward to the Saboyama group.
7. Saboyama, 30 m.
8. Akiu, 115 m.
9. Moniwa, 45 m.

The Tatsunokuchi group is regarded as belonging to the Upper Miocene, the Upper Mizuho (8), from molluscan fauna (9) and mammalian remains (3).

According to NIINO (5) the Tertiary deposits near the Shiobara hot-spring district, Shioya-gori, province of Shimotsuke, are divided as follows:

1. Shiobara plant bed, Pleistocene (7); unconformable to the underlying Tertiary formations.
2. Sekiya group; unconformable to the next older group.
3. Kanomata-sawa group.
4. Kawaji group, Paleozoic.

The Kanomata-sawa group almost certainly corresponds to the Tatsunokuchi group in age from molluscan fauna (11). The other locality of *Sequoia sempervirens* Endlicher fossilis is Tateyama-shita of Iwayado-machi in Esashi-gori, province of Rikutiu. This is not yet fully investigated, either stratigraphically or paleontologically.

From the geological occurrences it appears that *Sequoia sempervirens* was widely distributed during the Miocene, but it was absent in Japan in the Middle Pliocene.

The writer wishes to express sincere thanks to Professor H. YABE for his valuable advice and assistance during the present study, and also to Mr. M. TAKAMATSU for assistance with the illustrations.

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A CRETACEOUS SWEET GUM¹

ROLAND W. BROWN

(WITH ONE FIGURE)

During the latter part of June, 1930, the writer visited a fossil plant locality about 20 miles north of Kemmerer, Wyoming, at the junction of the northeastwardly flowing Everly Creek and Fontanelle Creek which comes down from the north and northwest. At this point the walls of the narrow gorge of Everly Creek and the south cliff facing the bend in Fontanelle Creek display a considerable section of the Upper Cretaceous Aspen shale, which approximates 1000 feet in thickness. The term shale as applied to the Aspen of this section includes strata of black and gray shales, clay, thin coals, bentonite, gray sandstone, and light-colored tuff, all dipping 35° westward and striking almost due north and south. The gray shales weather into long rounded hills with a distinctive greenish gray or silver gray appearance. The fossil plants are imbedded in the uppermost 125 feet of the formation, in a thin stratum of bluish gray, hard mudstone which is very brittle and fractures conchoidally. The plants are disposed at all angles through the matrix, making it somewhat difficult to get entire specimens. They are very well preserved and stand out black against the bluish gray background.

Of the fourteen species of plants in the collection,² the most attractive and suggestive form is one that I have called *Liquidambar fontanella*. Figure 1 illustrates the largest fragment of this species, but there were also in the collection numerous other fragments which permit the reconstruction of the entire leaf as given. So far as the remains permit a conclusion, all these leaves were 3-lobed, whereas the range of variation in modern sweet gums includes 3 to 7-lobed leaves, with the 5-lobed leaf the usual and most common form.

The breadth of the *Liquidambar fontanella* leaf from tip to tip of

¹ Published with the permission of the Director of the United States Geological Survey.

² BROWN, R. W., Fossil plants from the Aspen shales of southwestern Wyoming. U.S. Nat. Museum Proc. (In press.)

the lower lobes is 13 cm., and the length from the top of the petiole to the tip of the middle lobe is 8 cm. The lobes are 1 cm. wide near the base and become narrowly attenuate to their tips. The margin is finely crenate-serrate. The base is slightly cordate. The total length of the petiole is unknown. Three strong veins arising from the top of the petiole comprise the primary palmate venation, the laterals diverging at an angle of 60° from the middle vein. Eight to ten pairs of secondaries, appropriately spaced, branch off from the

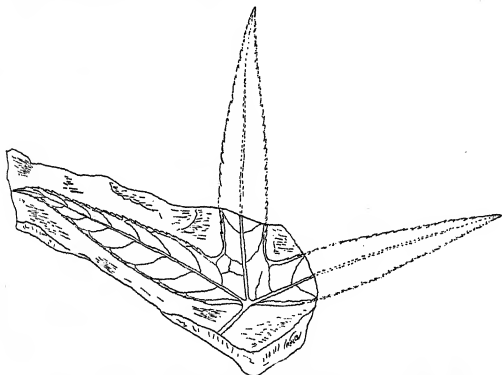


FIG. 1.—Reconstruction of *Liquidambar fontanella* Brown. $\times \frac{2}{3}$

primaries at approximately 60° , loop upward near the margin, and connect with the secondaries above. No finer details are discernible with a hand lens, and since no part of the epidermal or cuticular substance of the leaf remains, no microscopic studies can be applied. In general the characters of this leaf compare with none better than with those of the modern sweet gum leaf. The fossils differ chiefly in having unusually long lobes and a more restricted area of the basal region. From an esthetic point of view, the tree which bore these delicately graceful leaves was undoubtedly a striking object in the Cretaceous landscape along the stream courses and moist low country of southwestern Wyoming.

No remains of the characteristic *Liquidambar* fruits, called sweet gum balls, were found with the fossil leaves. This lack is regrettable, and it suggests the propriety of further search in the Aspen shales for these distinctive fruits, as well as for petrified wood and flowers, which would tend to confirm the determination of the leaves. Perhaps the identification as it now stands may be regarded by some as merely conjectural. VELENOVSKY (6), for example, has described and figured some 3-lobed leaves, called *Aralia formosa* Heer, from the Cretaceous of Bohemia. These, while superficially resembling *Liquidambar fontanella*, differ markedly in venation and in having a wedge-shaped rather than a cordate base.

A review (1) of the fossil sweet gums shows that some 20 Eurasian and 13 American species have been described, mostly from Tertiary and recent deposits. These remains include unmistakable flowers, fruits, and leaves. The earliest sweet gum was reported by HEER (3) from the Upper Cretaceous Dakota sandstone of Kansas. This leaf, called *Liquidambar obtusilobatus* (Heer) Hollick (5), as well as several other Cretaceous species, had the general aspect of a sweet gum leaf except that the margin was entire, a character which is now thought to ally it with *Sterculia* or *Aralia* rather than with *Liquidambar*. In view of these discredited Cretaceous species, the form now under discussion, *Liquidambar fontanella* Brown, becomes the earliest known sweet gum, or at least the earliest with leaves having crenate-serrate margins.

Whether *Liquidambar* originated from angiospermous ancestors with entire-margined leaves is a question. So far as I know, the study of juvenile, atavistic, or teratologic leaves of the living sweet gums does not reveal any with entire margins. HOLM (4), in a study of the leaf variations of the eastern American *L. styraciflua* Linné, showed that the leaves, shade leaves particularly, on seedlings were frequently entire, ovate, sometimes slightly lobed, but in all cases with toothed margins.

Sweet gum remains are well known from the American Cenozoic deposits. *L. callarche* Cockerell is present in the oil shales of the middle Eocene Green River formation, occurring in the contiguous corners of Colorado, Wyoming, and Utah. *L. californicum* Lesquereux from the auriferous gravels of California; *L. convexum* Cockerell

from the lake beds of Florissant, Colorado; and *L. pachyphyllum* Knowlton from the Mascall formation of the John Day Basin, Oregon, are recognized Miocene species. From the Pleistocene deposits of the Pamlico ("Chowan") formation near Seven Springs, Wayne County, North Carolina, BERRY (2) has recorded the fruits and leaves of the living *L. styraciflua* Linné.

The most familiar European fossil species is *L. europaeum* Al. Braun, from various Miocene localities, but principally from the celebrated lake beds at Oeningen, in Baden, Germany. There were, however, sweet gums in the Eocene and also in the later Tertiary of Europe, eastern Asia, and Japan. In short, the distribution of the genus *Liquidambar* was holarctic during Tertiary time, but during the Pleistocene glaciation many species were exterminated and the present restricted distribution of the four living species was brought about: one in central and southeastern China and Formosa; one in Asia Minor; one in southeastern United States and Mexico; and one in the uplands of Guatemala.

In what region of the world the genus *Liquidambar* became differentiated as such from its ancestors and relatives; how its distinct species were evolved; and by what routes and for what causes the species distributed are questions inviting speculation, but to which there appear to be no definite answers at the present writing. No small part of the difficulty here lies in the fact that there is no satisfactory method for distinguishing the fossil liquidambars on the basis of foliar remains alone. Distinction of species is largely by inference, which suggests that apparently similar leaves found in strata differing in age by a million years would most likely be recognized as distinct species if the trees on which they grew could now be placed side by side for comparison in their entirety.

Summary

1. *Liquidambar fontanella* Brown, together with thirteen other fossil plants, was obtained in a collection from the Upper Cretaceous Aspen shale in southwestern Wyoming.

2. The foliar characters of *L. fontanella* are almost identical with those of the living sweet gums, and justify its tentative assignment to the genus *Liquidambar*.

3. The genus *Liquidambar* achieved a holarctic distribution in Tertiary times, but during the Pleistocene glaciation was reduced to four species with restricted distribution.

4. The relation between *L. fontanella* and all other sweet gums, fossil and living, is conjectural; but it seems that *L. fontanella* is the earliest recognizable sweet gum which resembles living species.

UNITED STATES GEOLOGICAL SURVEY
WASHINGTON, D.C.

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"GEO-GROWTH" REACTION OF ROOTS OF LUPINUS

A. E. NAVEZ

(WITH ONE FIGURE)

A recent paper by KEEBLE, NELSON, and SNOW¹ has raised again the question of the existence of a geo-growth reaction in roots. Working with *Zea mays*, they reported that roots placed horizontally increased their rate of growth by as much as 50 per cent when measurements were made over periods of about 6 hours. They stated also that roots of *Pisum*, although not giving as clear-cut results, showed the same definite reaction. As the writer could not find such a geo-growth reaction in coleoptiles of *Avena*,² it was thought well to check the observations recorded by the British investigators, especially in view of the fact that tropistic movements of roots of *Lupinus* had been studied without such a reaction having been noticed.

During the past summer, the time-elongation curve was followed with a great number of roots of seedlings of *Lupinus albus* grown in moist sawdust at 22°-23° C. Measurements were made at first by means of a finely divided ruler; later by measuring with a curvimeter the enlarged photographic image of the roots kept undisturbed in their growing chamber. This latter process of measurement is simple with the aid of a camera with double extension and an objective which can be demounted, and whose back half can be used to give an enlargement of about twice that of the complete optical system. Outlines on light tracing paper can be taken very rapidly and with considerable precision.

Elongation of the normal, vertical root was found to proceed at constant rate between 30 and 90 hours after the start of germination. On the average, the roots during this time grew from 10 mm. to 50 mm. in length. The seedlings were grown in very moist sawdust and kept on this medium between periods of measurements, at which brief times they were transferred to a covered glass cell containing about 1 cm. of water, and lined almost completely, except

¹ KEEBLE, E., NELSON, M. G., and SNOW, R., Proc. Roy. Soc. B. 108:360. 1931.

² NAVEZ, A. E., and ROBINSON, T. W., Jour. Gen. Physiol. 16:133. 1932.

for two small openings at the front and back, with water-soaked blotting paper. Occasionally seedlings were maintained for longer durations in the observation cell. It should be noted that under all circumstances the roots were fully turgid, and could be kept growing at constant rate for periods up to 8 and 10 hours in a saturated atmosphere, when handled as here described. Care was taken to remove any adhering particles of sawdust and the excess water by means of a fine dry brush.

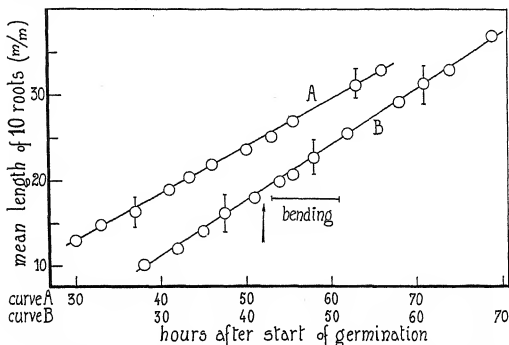


FIG. 1.—Time-mean elongation curve of roots of: A, 10 seedlings kept vertically throughout the experiment; B, 10 seedlings kept vertically, then turned horizontally (moment of rotation marked by arrow). Both series of observation points are best fitted by straight lines. For both curves the length of the short verticals above and below a few of the observation points indicates the extremes among the lengths averaged. The difference in the slopes of curves A and B results from the different temperatures (20.3° and 22° C.) at which they were obtained.

Under these conditions, as is seen in figure 1, the statement of KEEBLE and his co-workers cannot be confirmed: no geo-growth reaction of the root was shown by these seedlings. The behavior of the roots they observed must be accounted for in some other way. Of course one could think that the difference in material might explain the difference in results. At the time of the present experiments, no material of *Zea mays* was available. More recently an experiment was performed with *Z. mays*, however, involving 48 seeds, and the phenomenon was not detectable.

There is the possibility of error resulting from the technique used by KEEBLE and his co-workers. In brief, their method was as follows: an outline of the root was made on which a thread was laid, along the middle line of the drawing; the length of the root was then known by transfer of the thread to a graduated scale. The method of expressing the measurements in terms of percentage of growth of the straight root may not seem conducive to a high degree of accuracy; but, disregarding these possible errors, the differences recorded are of sufficient magnitude that they cannot be attributed entirely to the technique of measurement and to the method of expressing these measurements.

The cause of divergence, therefore, must probably reside in the difference of experimental technique. It had been noted that seedlings in the vertical position showed a reduced rate of growth when a drop of water was hanging at the tip of the root. It was easy to show that this drop of water, even when small, would retard elongation of the root, and that when it was removed normal elongation was resumed.

In the case of a root placed horizontally, the presence of a drop of water at the very tip is almost impossible; it would spread rapidly in a film over the apical region with a definite excess on the lower side of the horizontal root. This, of course, would make the conditions of growth different for the two positions, and results such as those reported by KEEBLE and his collaborators could be expected. In cases in which the roots have the excess water removed, no difference in the rate of elongation can be found.

While this paper was being prepared for publication, CHOLODNY³ published a series of observations on the growth of roots of *Zea mays* which are in perfect agreement with the views here expressed.

It would seem logical to ascribe to a technical imperfection the results reported by KEEBLE, NELSON, and SNOW, and to maintain until further adequate study that roots of *Lupinus albus* and *Zea*, as well as coleoptiles of *Avena*, do not show increased rate of elongation during geotropic response.

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³ CHOLODNY, N., Arch. Wiss. Bot. (Planta) 17:794. 1932.

BRIEFER ARTICLES

PARTIAL PHYLLODY OF *YUCCA ELATA*

(WITH ONE FIGURE)

Observations on phyllody in *Yucca elata* Engelm., previously reported from the Jornada Experimental Range in southern New Mexico, dealt with specimens in which differentiation of flowers failed completely.¹ All of the cases found from 1926 to 1931 were of this type. In July, 1932, however, three specimens of partial phyllody were noticed. These specimens of partial phyllody, two of which are shown in figure 1, were produced in 1931, probably during the early summer. When they were found a year later, the panicles were dry, the capsules open, and most of the seeds were dispersed. Even though the stalks were a year old, they were sufficiently well preserved to demonstrate this unusual feature for *Y. elata*.

The usual inflorescence of *Y. elata* is a much branched panicle with perfect flowers. It has been reported previously² that the number of blossoms on different flower stalks may vary from 75 to sometimes more than 200, but the number of flowers which produce mature capsules is low, rarely as high as 30 per cent. Observation of the flower stalks shown in figure 1 revealed that flowers and mature capsules were produced on the lower branches of the panicles, but flowers failed to differentiate on the upper portions, thus producing the condition of partial phyllody. The smaller panicle produced nine normal branches, supporting approximately 50 flowers; five branches showing transition between typical growth and phyllody; 14 leaf clusters on the main axis and a dense terminal group of leaf clusters. Five of the flowers produced mature capsules. The larger panicle (fig. 1) produced 25 normal branches, with approximately 100 flowers, six transitional branches, nine branches in condition of phyllody, and the terminal leaf cluster. Eleven mature capsules were produced on this specimen.

It is impossible to determine the cause of the condition observed from the scanty material available. Both of the specimens were inhabited by the carpenter bee, *Xylocopa californica*, but this insect does not enter

¹ CAMPBELL, R. S., A case of phyllody in *Yucca elata*. BOT. GAZ. 88:109-110. 1929.

² CAMPBELL, R. S., and KELLER, J. G., Growth and reproduction of *Yucca elata*. Ecology 13:364-374. 1932.

Yucca flower stalks until after the flowers are fully developed and it is by no means confined to panicles in a condition of phyllody. The specimens shown in figure 1 were taken from stalks with a connecting underground stem.

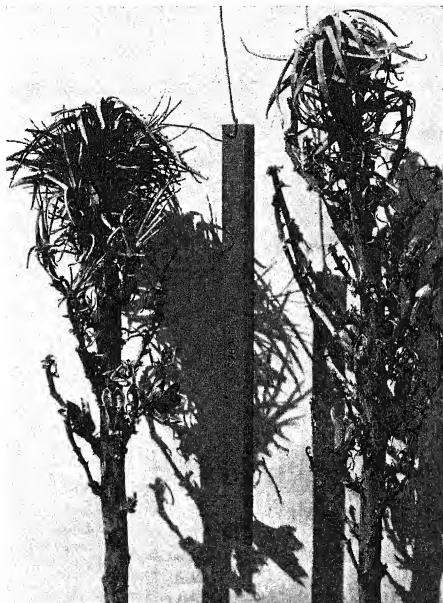


FIG. 1.—Two flower stalks of *Yucca elata*, showing partial phyllody

Although many observations have been made, no cases of phyllody have been observed in other species of *Yucca* found in southern New Mexico, *Y. baccata*, *Y. glauca*, and *Y. macrocarpa*.—R. S. CAMPBELL and J. G. KELLER, *Southwestern Forest and Range Experiment Station, U.S. Forest Service, Las Cruces, New Mexico.*

AN ADAPTER FOR PHOTOMICROGRAPHY
WITH BOX CAMERAS

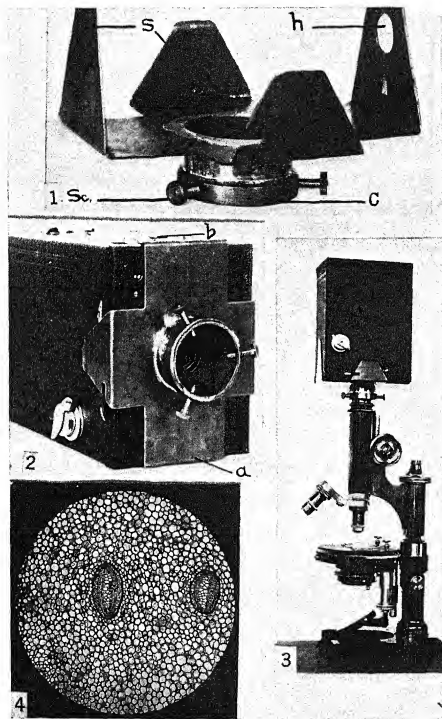
(WITH FOUR FIGURES)

The use of such a cheap box camera as a Kodak is perhaps rare in the field of photomicrography. With such a camera, however, photographs can be taken with great ease and precision, and with considerable economy of time. The process is simple, consisting in taking the picture of a focused object by fixing the opening of the camera directly on the eye-piece of the microscope. With a time exposure, however, it is difficult to hold the camera steadily for the time required. To remove this difficulty, a simple piece of apparatus has been devised which works very satisfactorily.

The adapter is made of a brass sheet about 1 mm. thick. It consists of a collar (length 20 mm., inner diameter 32 mm.) soldered to the central hole (diameter 34 mm.) of a brass plate cut in the form of a cross (figs. 1, 2). The collar is provided with three screws. A portion of each limb of the brass plate is bent at right angles to its central part. Provision is made, by proper trimmings and holes in the bent limbs, for the operation of the shutter, time-exposure bar, etc., without interference when the adapter is fixed on to the camera.

The object to be photomicrographed is first correctly focused under critical illumination of the microscope. The adapter is then slipped over the face of a loaded camera, so that it grips the four sides of the box rather firmly. The time exposure bar is pulled out, the apparatus adjusted to bring the opening of the camera flush with the surface of the eye-piece of the microscope, and the collar screws tightened (fig. 3).

The time exposure will vary, of course, with the intensity of light and the kind of plate or film used. With an ordinary film or plate, and with the iris-diaphragm of the microscope open full, an exposure of about two seconds suffices in the ordinary daylight of the laboratory. A specimen of a photomicrograph (a transverse section of the rhizome of *Leucostegia hymenophylla*) taken with this arrangement is reproduced in figure 4. The depth of focus obtained is even throughout the field and no blurring is noticeable toward the periphery, as is usually the case in most photomicrographs.—T. C. N. SINGH, *Botany Department, Ravenshaw College, Cuttack, India.*



FIGS. 1-4.—Fig. 1, the adapter: *c*, collar; *s*, vertical sides; *sc*, screw; *h*, hole for handle button of camera. Fig. 2, camera with adapter in place. Fig. 3, camera adjusted to microscope. Fig. 4, photomicrograph of transverse section of rhizome of *Leucostegia hymenophylla* taken with the arrangement as shown in fig. 3.

CURRENT LITERATURE

English edition of Braun-Blanquet's *Pflanzensoziologie*

A recent author has characterized ecology as "the science of communities." Whether plant ecologists generally will accept this restricted definition of their field is extremely doubtful; among English speaking botanists, at any rate, long usage has firmly implanted the idea of plant ecology as the study both of plant communities and of individual plants in their relation to environment. Be this as it may, there can be no question that the study of plant communities, or plant sociology, if not the most important, constitutes at any rate the most unique phase of plant ecology, the phase in which it is most distinct from all other fields of plant science. The present volume¹ is one of the very few modern textbooks which deals specifically with the subject of plant sociology.

As described by the author, the field of plant sociology embraces all knowledge of vegetation in the widest sense; it includes all phenomena which touch upon the life of plants growing in social units. It has to do with the investigation of plant communities with reference to their organization, structure, and composition, their dependence upon one another and upon environment (synecology), their developmental relations (syngenetics), their geographical distribution (synchorology), and their classification (systematics). These diverse points of view form the basis of treatment in the text, which is divided into five principal sections, one for each of these heads, in addition to an introductory section on social life among plants. A review of the original German edition has already appeared in these pages.² It should be emphasized again, however, that the book is of special interest by reason of the attention it directs to various concepts and phases of plant community study which have been highly developed in continental Europe while largely neglected in this country; in particular, to the structural features of the community and the methods by which these features can be studied, evaluated, and coordinated. In this respect the book is outstanding in its field. Of special note also is the clear and extended discussion of the habitat factors, which are classified from a distinctly ecological point of view into six groups, namely, climatic factors, edaphic or soil factors, soil organisms, soil types, orographic factors, and biotic factors.

In the preparation of the English edition the text has been thoroughly revised, and in addition various topics have been introduced which did not appear in the original German edition. In its present form, thanks to the combined

¹ BRAUN-BLANQUET, J., *Plant sociology*. Translated, revised, and edited by FULLER, GEORGE D., and CONARD, HENRY S. pp. xviii+439. figs. 180. Bibliography. McGraw-Hill Book Co. New York. 1932.

² BOT. GAZ. 88:451, 452. 1929.

skill of author and editors, the book represents a comprehensive, well-balanced, and scholarly exposition of the science of plant communities, constituting a notable achievement in the progress of plant ecology.—G. E. NICHOLS.

Handbook of soil science

Beginning in 1929, there have appeared at intervals the various volumes of the handbook of soil science.³ This work is edited by E. BLANCK of the University of Göttingen. He is also the author of a number of sections and has been extensively aided by other German soil scientists.

In nature and content, the volumes may be divided into two groups. The first seven are concerned with general features of soil science. The processes of soil formation and the various factors involved are discussed in detail. The various soil types are described and their geographical distribution given. There is a full discussion of the physical, chemical, and biological characteristics of the soil. The last three volumes deal with special or applied features of soil science. In these volumes are discussed such subjects as: methods of determining the fertility of the soil; principles of soil tillage; manuring the soil; and methods of modifying the activity of soil microorganisms. The last volume discusses the technical uses of the soil.

Taken as a whole, the work is an excellent and comprehensive treatise of the entire field of soil science, both pure and applied. The ten volumes comprise a total of nearly 5000 pages and there are over 600 illustrations. The handbook should be of great help to all interested in this subject.—S. V. EATON.

Environmental factors

Realizing the impossibility of keeping familiar with the increasing multitude of scientific publications, the ecologists and pathologists will welcome a bibliography.⁴ This pamphlet includes 3689 citations arranged alphabetically, with three classified indexes of environmental factors, host-disease injury, and hosts respectively. Its usefulness is unquestionable.—G. D. FULLER.

Shrubs of Indiana

As a companion volume to the *Trees of Indiana* recently noted in this journal,⁵ DEAM has now written a similar volume on the shrubs.⁶ Its most valuable features are the distribution maps, the excellent illustrations, and the ecological notes. The arrangement of the material, the details of peculiar distribution, the quality of the illustrations, and the excellence of the typographical work all make it a model that may well be followed.—G. D. FULLER.

³ BLANCK, E., Editor, *Handbuch der Bodenlehre*. Vols. I-X. Hirschwaldsche Buchhandlung, Berlin. 1929-1932.

⁴ WILSON, J. D., *Environmental factors in relation to plant disease and injury: A bibliography*. Ohio Agric. Exp. Station Technical Series. Bull. 9. pp. 203. Wooster, Ohio. \$1.00. 1932.

⁵ BOT. GAZ. 93:492-493. 1932.

⁶ DEAM, CHAS. C., *Shrubs of Indiana*. Dept. of Conservation, State of Indiana, Publ. 44. 380 pp. 155 maps. 153 pl. 1932.

THE BOTANICAL GAZETTE

June 1933

RELATION OF OXYGEN PRESSURE AND TEMPERATURE TO THE INFLUENCE OF ETHYLENE ON CARBON- DIOXIDE PRODUCTION AND ON SHOOT ELONGA- TION IN VERY YOUNG WHEAT SEEDLINGS^{*}

WARREN B. MACK AND BURTON E. LIVINGSTON

(WITH FIVE FIGURES)

Ethylene influence on plant processes; review of literature

That illuminating gas is injurious and often fatal to plants was reported by GIRARDIN (16) in 1864. Other observers, among whom were NELJUBOW (34, 35), KNY (28), VIRCHOW (57), SPÄTH and MEYER (51), MOLISCH (33), STONE (52, 53), and WILCOX (62), described symptoms of plants injured by illuminating gas or laboratory air. Some of the more striking symptoms described were wilting, yellowing, and falling of leaves; browning and death of cambium in trunks of shade trees and conspicuous fungous growth on injured trunks; increased diameter and characteristic bending of roots of maize seedlings; formation of enlarged lenticels on willow twigs in humid atmosphere; and loss of negative geotropism in stems of certain seedlings, including wheat. CROCKER and KNIGHT (4) discovered that minute traces of illuminating gas in the atmosphere prevented the opening of carnation flower buds, and demonstrated that ethylene was the most active toxic agent in causing this injury.

^{*} Botanical contribution from the Johns Hopkins University, no. 116. Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper no. 565.

CROCKER, KNIGHT, and ROSE (5) showed that very small concentrations of ethylene in the air might produce characteristic reactions, particularly in etiolated seedlings of sweet pea. In an atmosphere containing one volume of ethylene in a million of air, etiolated sweet pea seedlings had thicker and shorter stems than other like seedlings that had not been exposed to ethylene, and the affected seedlings failed to exhibit the usual negative geotropism of epicotyls. KNIGHT and CROCKER (26) noted similar effects of tobacco smoke and attributed these effects to ethylene contained in the smoke. HARVEY and ROSE (20) discovered that seedling roots of species of *Hibiscus*, *Syringa*, *Ailanthus*, *Croton*, *Diervilla*, *Ricinus*, and *Ulmus*, when treated with ethylene, showed sloughing off of the cork tissue and great proliferation of other tissues from the phloem outward. HARVEY (19) found that sweet pea seedlings injured by ethylene contained relatively larger amounts of simple soluble compounds and relatively smaller amounts of more complex soluble compounds and insoluble ones than were present in similar seedlings that had not been exposed to ethylene. The osmotic values and the permeability of ethylene-treated tissues were somewhat greater than those of untreated tissues and respiration was somewhat less rapid, except for a temporary acceleration of CO₂ production that occurred during the first three hours of exposure to ethylene.

SORAUER (50) confirmed earlier reports that treatment with illuminating gas might cause leaf yellowing, leaf fall, and failure of flower buds to open, but he attributed these effects to inadequate oxygen supply. WEHMER (61) observed similar effects of illuminating gas acting as a poison, and found that the stage of development of the plant and its state of health were important in determining the extent of the gas influence. DOUBT (13) observed that plants of *Coleus*, *Ricinus*, *Datura*, and *Mimosa* lost their leaves after having been exposed to illuminating gas for a few hours. *Mimosa* lost its sensitiveness to touch before other forms of injury were pronounced. She found also that illuminating gas or ethylene caused proliferation of twigs of apple and pear, especially in lenticels and leaf scars. GOODSPEED, MCGEE, and HODGSON (17), and also KENDALL (25), reported that CO, CO₂, and ethylene (all commonly present in il-

luminating gas) each caused premature abscission of flowers and fruits in forms of *Nicotiana* and *Citrus*. SCHWARTZ (47) confirmed reports of DOUBT and other earlier observers that petioles of *Helianthus*, *Lycopersicum*, and *Salvia* showed epinastic curvatures in the presence of illuminating gas.

WALLACE (58, 59, 60) investigated internal and external conditions influencing the formation of intumescences on apple twigs in the presence of ethylene, and observed that air humidity had no influence in his experiments, but that temperature and oxygen content of the atmosphere were influential. Between the limits of 75% of ethylene and 25% of oxygen at one extreme and one part of ethylene in 10,000 of air at the other, the concentration of ethylene made little difference in the nature or degree of the effects produced; concentrations as low as one volume of ethylene in 100 million volumes of air caused some formation of intumescences.

PRIESTLEY (37, 38) pointed out that etiolated pea seedlings have in health a well-marked Casparian strip in the primary endodermis, extending from the base of the stem nearly to the stem apex, but that this strip failed to form under the influence of ethylene. He suggested that this influence of ethylene might be due to increased mobility of unsaturated fatty acids.

The commercial use of ethylene to hasten the advent of an edible condition in fruits and vegetables and to shorten the dormant period in resting plant organs has been recently developed from the work of many experimenters. CHACE and DENNY (1) reported that the yellowing of citrus fruits could be accelerated by exposing them to an atmosphere of one volume of ethylene in 5000 or more volumes of air. To hasten the blanching of celery, HARVEY (22) recommended the use of one volume of ethylene in from 1000 to 10,000 volumes of air; he suggested later (23) that the same treatment might be used to accelerate ripening in bananas, pineapples, and other tropical fruits. ROSA (43) found that ethylene hastened the ripening of tomatoes, and he afterwards (44) reported that propylene was more effective. CHACE and SORBER (3) recommended the use of ethylene to promote the softening of Bartlett pears; and CHACE and CHURCH (2) and DAVIS and CHURCH (6) found that ethylene treatment hastened coloring, softening, and loss of astringency in persimmons.

McCALLUM (30) was able to shorten the period of dormancy of potato tubers by brief treatment with ethylene compounds and related substances. ROSA (45, 46) found that potato tubers that had been treated with ethylene sprouted earlier and produced an unusually large number of sprouts from each eye. VACHA and HARVEY (56) observed that a 6-day exposure to one volume of ethylene in 1000 volumes of air caused more rapid sprouting in Bliss Triumph and Russet Burbank potato tubers, but that other potato varieties tested by them were not perceptibly affected. They found also that ethylene, and propylene also, hastened the sprouting of gladiolus corms and quickly broke the dormancy of hardwood cuttings of apple, pear, plum, cherry, mock-orange, and honeysuckle. Both gases accelerated the germination of seeds of buckthorn, high-bush cranberry, Tartarian honeysuckle, and snowberry. HABER (18) reported that a 24-hour exposure of onion sets to ethylene-air mixtures containing one part of ethylene to 100 or 200 parts of air accelerated early growth and resulted in unusually large bulbs. Narcissus bulbs matured earlier when treated with an ethylene-air mixture of one part of ethylene to 200 or 300 parts of air. Air humidity appeared to be influential, for the lower concentration of ethylene was as effective with high relative humidity as the higher concentration was with very low relative humidity. HABER stated that when sweet-corn kernels were soaked for 24 or 48 hours in a solution formed by bubbling ethylene through water, they germinated more rapidly than similar seeds soaked in untreated water for the same length of time and at the same temperature. Plants from ethylene-treated seeds produced edible ears earlier than did those from untreated seeds.

Not all investigators have found ethylene to be uniformly effective to break dormancy or hasten the processes of ripening and maturing in plant organs. Earlier experiments of CHACE and CHURCH (2) showed but little effect from the use of ethylene for hastening the ripening of tomatoes; but their later experiments, in which degree of maturity of the fruits was taken into account, showed relatively small but notable effects. They found also that ethylene treatment did not alter the percentage of moisture, reducing sugars, sucrose, citric acid, or pentosans in citrus fruits, although it did accelerate

coloring. Treatment of avocados with ethylene caused the development of a brassy color of the skin and a darkening of the flesh. WORK (63) observed but little effect of ethylene in the ripening of tomatoes, excepting that fruits in the green-mature stage ripened more promptly after treatment with this gas. ROBBINS (42) found no advantage in the use of ethylene to hasten the blanching of celery, either in a tight compartment or in a refrigerator car. He also reported that ethylene treatment gave no acceleration of ripening in Marglobe and Greater Baltimore tomatoes with temperature ranges of 55°-65°, 68°-70°, or 70°-75° F. KOHMAN (29) reported that ethylene treatment was of no value for treating tomatoes to be used for canning. DENNY (9, 10) found that ethylene was ineffective in terminating the dormancy of potato tubers, with air mixtures containing ethylene at concentrations between 5 and 150,000 volumes per million volumes of air. He reported (12) that exposure for 12 days to a 1:1000 ethylene-air mixture failed to shorten the rest period of freshly harvested gladiolus corms, although this treatment was effective on corms one month after harvest. Similarly, ethylene treatment was ineffective in breaking the dormancy of pot-grown lilacs and crabapples (11), although it was effective on forms with less pronounced dormancy, such as *Prunus triloba*. DENNY observed also (7) that little or no acceleration of the yellowing process occurred in lemons at temperatures below 45° or above 92° F., or if the oxygen concentration were very low or the ethylene concentration were above 80%. Although HIBBARD (24) found that ethylene hastened destruction of chlorophyll in celery, oranges, and apples, he did not recommend its use for hastening the blanching of celery or for ripening bananas. FULTON, STEVENS, and WOOTTEN (15) stated that oranges should be nearly mature for ethylene treatment to be effective; with immature fruit, too high temperature, or too great concentration of ethylene, serious damage might result from ethylene treatment.

Several of the studies just mentioned dealt to some extent with physiological questions other than those of immediate practical interest. DENNY (8) reported that treatment with ethylene at concentrations of from one to 1000 volumes in a million volumes of air caused increases of from 100 to 250% in the rate of CO₂ production

by lemons. With bananas, REGEIMBAL, VACHA, and HARVEY (40) observed increases in the rate of CO_2 production as great as 150% within 15 or 20 minutes after the fruits were exposed to a mixture of ethylene and air containing one volume of ethylene in a thousand. This remarkable acceleration disappeared entirely in half an hour if no more ethylene was introduced into the respiration chamber, but a second and third dose of ethylene resulted in similar brief outbursts. The same investigators reported that bananas ripened with ethylene contained 20-25% more sugar than those ripened by ordinary methods. MACK (31) observed increased CO_2 production and more rapid decay in celery plants treated with ethylene than in otherwise similar but untreated plants. DAVIS and CHURCH (6) found that CO_2 production by ripening persimmons was accelerated by exposure to a gas mixture of one volume of ethylene to 1000 volumes of air.

REGEIMBAL and HARVEY (41) observed that activity of the proteolytic enzym bromelin was somewhat greater in juice from ethylene-ripened pineapples than in juice from untreated fruits, but the treated pineapples were said to have been somewhat riper than the others at the time treatment was applied, and it is at least possible that this difference in bromelin activity may not have been due to ethylene treatment. ENGLIS and ZANNIS (14) found that ethylene had no effect on the activity of diastase and invertase in their experiments. REA and MULLINIX (39) reported that exposure to ethylene caused maize and wheat starch to be transformed into sugar, both in the dry state and in suspensions containing 1 gm. of starch in 100 ml. of water.

From this incomplete review it is clear that our present knowledge of ethylene influence on plants and plant substances is so fragmentary that satisfactory general statements about ethylene relations are not yet possible. There seems to be no doubt that ethylene has sometimes produced stimulation and sometimes retardation, and that it has sometimes failed to exert any notable influence on the processes considered. Experimentation has been predominantly of a preliminary and necessarily somewhat naïve nature, and most of the experimental results that have been published must be considered as merely isolated facts, many of which appear to be discordant. Constructive comparisons are generally impossible, either

because the plant material employed for different tests has differed in unknown ways or because the experimental procedure has been such as to preclude satisfactory repetition and comparison. A considerable part of the experimentation in this field has been planned with reference to technological aims, with a desire to establish commercially useful methods for ripening fruits, awakening dormant tissues, and the like, rather than to advance fundamental knowledge. An apparent desire to encourage the use of ethylene in the commercial handling of horticultural products is detectable in some recent publications on ethylene effects, but that does not necessarily detract from the scientific value of the recorded observations. On the other hand, discrepant results apparently due to fundamentally unsatisfactory logical or experimental technique not only tend to retard practical applications but also do not, in themselves, greatly accelerate the advance of scientific knowledge.

The general subject of chemical stimulation and toxicity occupies a prominent position in plant physiology and its many applications. Consistently and adequately planned experimental studies in this field, which are becoming relatively more feasible as the concept of multiple causation or determination becomes more widely appreciated, are greatly needed to advance our scientific grasp of the control of vital processes in general. The number of chemical compounds that call for consistent investigation in this connection is great, for almost every compound would doubtless exert some influence if applied to plants in suitable concentrations or at suitable rates of supply, with adequately chosen time periods and background complexes. Aside from simple convenience with regard to experimental technique, it would make little difference what compound or compounds might be selected for studies of this sort. Ethylene, however, lends itself rather readily to experimentation with several experimental variables, and it is clear that results secured from experiments with this substance may throw light on questions of horticultural importance as well as on important aspects of physiological science.

Preliminary experimentation

The studies reported in this paper were an outgrowth from a few practical tests concerning the value of ethylene as an agent for

hastening the blanching of celery, as recommended by HARVEY (22). They were begun as a series of preliminary experiments planned for orientation. Some of the results with celery have been reported previously (31) and others will be presented later in this paper. Additional preliminary experiments were also performed with material other than celery. Many valuable suggestions were received from Professor D. E. HALEY of the Pennsylvania State College, where the preliminary work was done.

The present section is devoted to the preliminary experiments and the following section reports a more elaborate study, on wheat seedlings.

In the first celery tests, some of the results of which have been reported (31), galvanized sheet-iron cylinders with a capacity of about 120 liters were used as containers. Each was made gas-tight by means of a broad rubber band fitting tightly around the top and partly over the edge of the lid. Gases were admitted or withdrawn by means of two metal tubes soldered into holes in each lid, the tubes being ordinarily closed by means of short pieces of rubber tubing and screw cocks. At the beginning of each experiment one, two, or three celery plants were inclosed in each container, in an atmosphere of ordinary air, and a volume of commercial ethylene sufficient to make up the desired initial gas concentration was then admitted from a gas burette. The volume of ethylene required was always so small that it could be added to the container without appreciable change in total gas pressure.

Observations on the rate of blanching were made from time to time through a glass plate which closed a suitable opening in the lid of one of the containers, and was ordinarily covered to exclude light. Final observations were made after removing the plants from all containers. Estimates of the average hourly rates of CO_2 production were made from measurements of the total amount of CO_2 that had accumulated in each container during the experimental period. These measurements were made by sweeping out the accumulated CO_2 with a rapid stream of CO_2 -free air, absorbing the CO_2 in soda-lime and weighing.

In these experiments, blanching was accelerated by ethylene in concentrations varying between one volume and 100 volumes in 50,000

volumes of air. Most rapid blanching occurred with the lower concentrations of ethylene. With the higher concentrations injury of the petioles was observed, splitting and peeling of the inner surface and development of pithiness. Decay occurred in many instances, its progress being most rapid with the lower concentrations of ethylene, which produced most rapid blanching. Carbon-dioxide production was most rapid when blanching and decay were most pronounced.

Additional preliminary experiments that are to be reported at this time dealt with the effect of ethylene on the catalase activity of celery plants, with immediate effects of ethylene on CO_2 production by celery and chard leaves, tomato fruits, and wheat seedlings, and with the influence of ethylene on catalase activity and oxidase activity in wheat seedlings.

EFFECTS OF ETHYLENE ON CATALASE ACTIVITY OF CELERY.—For the catalase tests, celery plants were sealed in separate containers, as already described, for periods of from 30 min. to 48 hr. At the start of each test the control chambers contained ordinary air and the others contained known mixtures of air and ethylene. Before each plant was placed in its chamber a sample of leaf tissues was taken and a similar sample was taken at the end of the test, and catalase activity was estimated for each sample. A sample consisted of five discs cut from a leaf by means of a 1-cm. cork borer. The five discs cut before placing the plants in their containers were taken from as many different leaflets of the same leaf, and those cut after treatment were from the same leaflets as had been used before, the two cuts being symmetrically placed on each side of a conspicuous vein. The five discs of each sample were ground, with their weight of powdered CaCO_3 , in 50 times their weight of distilled water, and the preparation was stored in a rubber-stoppered bottle for 24 hr. in an ordinary refrigerator. KNOTT (27) reported that this method of preparing leaf samples gave consistent results for catalase activity.

Relative catalase activity was measured according to the method described by KNOTT (27). Two ml. of leaf-tissue infusion were shaken with 2 ml. of standard H_2O_2 solution ("Dioxogen") neutralized with powdered CaCO_3 . The oxygen produced was measured at atmospheric pressure in a eudiometer and relative catalase activity was taken to be inversely proportional to the time required to produce a

standard volume of oxygen. Only small changes in catalase activity were found to have occurred during any of the experimental periods, and such changes as did occur were apparently not related to the concentration of ethylene at the start of the experiment; they may have been associated with small temperature differences. At any rate, no effect of ethylene on catalase activity was apparent.

IMMEDIATE EFFECTS OF ETHYLENE ON CO_2 PRODUCTION.—In the experiments on the immediate effects of ethylene, 4-liter tubulated bell-jars were used as experiment chambers. The bottom of each jar, through which the plant material was introduced, was closed by means of a circular glass plate held tightly in position by means of a broad rubber band. Each jar was provided with a rubber stopper bearing an inlet and an outlet tube, the inlet projecting only slightly below the stopper while the outlet extended nearly to the bottom of the jar. Each inlet tube forked just above the stopper, one branch leading from a series of wash bottles with KOH solution for the removal of CO_2 from the incoming air, while the other branch (provided with a glass stopcock for regulating delivery of ethylene) led to a reservoir of ethylene under slight pressure. The stopper was provided with several additional tubes, each closed by means of rubber tubing and screw cock, so that the gaseous contents of the jar might be confined or withdrawn at will, or a current of air might be passed continuously through the jar by means of a filter pump. Each outlet tube led to a Pettenkofer tube partly filled with glass beads and containing a measured amount of standard $\text{Ba}(\text{OH})_2$ solution, for absorbing the CO_2 given off by the plant material. During an experiment each jar was surrounded by water in a reservoir from which light was excluded. The water provided a seal and served to regulate the temperature.

At the beginning of each experiment the container was sealed for 15 or 25 min. and then the gas contents were swept out by means of a rapid current of air continuing for 5 min. Thus the CO_2 evolved by the plant material in 20 or 30 min. was removed and absorbed in the Pettenkofer tube, being finally measured by titration of the $\text{Ba}(\text{OH})_2$ solution with standard HCl. At the end of this 5-min. period the bell-jar was again sealed and the procedure repeated. Several successive measurements of the amount of CO_2 produced in 20 or in 30

min. were made in this manner, until a fairly constant rate of respiration was indicated. Ethylene was then introduced, in quantity sufficient to make the volumetric proportion of ethylene to air in the container either 1:400 or 1:800, and measurements of CO_2 production were continued as before. Exposure to ethylene was for only one period at a time, although the same material was treated with ethylene at several different times, usually separated by intervals greater than 1 hr.

Experiments of this sort were performed with celery and chard leaves and with tomato fruits. The rate of evolution of CO_2 was found to fluctuate somewhat from period to period but its fluctuations were apparently not related to the ethylene treatment in any consistent way. In some instances the rate was higher with ethylene than without it and in other instances it was lower with ethylene. With these materials, and with the procedure followed in these tests, the respiration rate was apparently not influenced by a 15-min. or a 25-min. exposure of the material to an ethylene-air mixture containing 400 or 800 volumes of air to one volume of ethylene.

PRELIMINARY EXPERIMENTS ON CELERY WITH MAINTAINED GASEOUS ENVIRONMENTS.—In the experiments already described the composition of the atmospheric environment was known only for the very beginning of an exposure. Changes in the oxygen and CO_2 content of the atmosphere must occur in experiments of this kind, becoming more pronounced as the exposure period is prolonged, and it is probable that the concentration of ethylene in the inclosed space decreases with time. With this probability in mind a method was adopted by which a known mixture of ethylene and CO_2 -free air was passed continuously through the experiment chamber, so that the composition of the inclosed atmosphere was maintained practically constant throughout the whole period of exposure. All the CO_2 given off was absorbed, being measured at intervals, and the experimental period was prolonged until changes in the appearance of the plant material were clearly evident.

The apparatus employed was much like that described in the preceding section. A telltale was introduced into each branch of the inlet tube just outside the bell-jar. The one in the air line was a small gas-washing bottle containing a weak solution of $\text{Ba}(\text{OH})_2$,

colored with phenolphthalein. All air entering the jar bubbled through this solution and this telltale gave assurance that CO_2 had been effectively removed from the entering air. The rate of air flow, which was about 800 cc. per min., was regulated by adjusting the water pressure in a filter pump used to draw air through the apparatus. The telltale in the ethylene-supply line consisted of a gas-washing bottle of water, through which the rate of gas flow (0.8 cc. per min.) was indicated by the number of bubbles passing in a given time. The two jars of a test were in the same water reservoir and the temperature was regulated from time to time by the addition of warm or cool water as might be required.

When an experiment was begun, three similar, nearly mature celery plants with roots removed were weighed and placed in each of the two bell-jars, with their bases in a small amount of water. An air stream without any ethylene was passed through each jar until repeated measurements showed that the ratio of the two rates of CO_2 production had become fairly constant. Then ethylene was admitted to the air stream for one jar, at such a rate that the volumetric proportion of ethylene and air passing through the jar was approximately 1 to 1000. The other jar continued to receive air only. Successive measurements were then made on the rates of CO_2 production by the treated and untreated plants, at intervals of a few hours at first and later at intervals as long as 12 hr. Each experiment was continued until blanching was well advanced in the untreated as well as in the treated plants.

Five experiments of this sort were performed, with temperature about $19^\circ \pm 3^\circ$. The length of the experimental period was over 100 hr. in every instance, generally from 100 to 150 hr., with observation intervals about 5 or 6 hr. long. Mean hourly rates of CO_2 production for the successive observation intervals were computed as for 100 gm. of plant material. The rate in the control chamber (without ethylene) fluctuated irregularly, between about 4 or 5 mg. and about 9 or 10 mg. per hour. From 40 to 70 hr. after the introduction of ethylene into the continuous gas stream for the experiment jar, the rate of CO_2 production in that jar began to exceed the corresponding rate in the control jar, in experiments 1, 2, 3, and 5 of this series. The acceleration became gradually greater during an additional 80-

120 hr., after which it gradually decreased. At the end of the experiment, in these instances, the mean hourly rate of CO_2 production was about the same for both jars. The treated plants showed irregular fluctuations in the rate of CO_2 production throughout the whole period, similar to those noted for the untreated ones; but a marked acceleration apparently due to ethylene was clearly shown in all four of these tests. These fluctuations were not related to the temperature fluctuations nor were they related to night and day periods. For the observation intervals in which the accelerating influence of ethylene was most pronounced, the rate with ethylene was generally 10-30% more rapid than the corresponding control rate.

Experiment 4 showed no ethylene acceleration of CO_2 production in a period of 125 hr.; indeed there was some evidence of a slight but approximately maintained ethylene retardation of this process throughout the whole period.

It appeared from these results that this ethylene treatment might be found: (a) to exert no effect on CO_2 production if the period of treatment were shorter than 2 or 3 days or longer than 5 or 6 days; (b) to accelerate CO_2 production if the length of period were between about 3 and about 6 days, acceleration increasing to a maximum value and then decreasing to zero; (c) to retard CO_2 production slightly, but continuously, almost from the beginning of the test. The single wholly discrepant result and the apparently pronounced influence of period length on the results from each of the four experiments that were in general agreement indicated that such influential conditions need to be understood more precisely than they were in these tests. The plants were more nearly mature for the later experiments of this series than for the earlier ones, but it is remarkable that the fourth, and not the last test, was the discrepant one.

In the four experiments that gave similar results, blanching of the leaves was first evident at about the same time in both jars, but it progressed somewhat more rapidly in the jar continuously supplied with ethylene. In experiment 4, however (which was discrepant with respect to ethylene effect on CO_2 production), blanching was more rapid in the untreated plants. The time required for blanching, or for a given stage of blanching, was shorter for the later experiments than for the earlier ones, presumably on account of differences in the

degree of maturity of the plants used. In both the treated and the untreated plants the outer (older) leaves became blanched most rapidly and the effect of ethylene to hasten blanching was more pronounced for these leaves than for the inner (younger) ones.

It was observed in several cases that the inner (younger) petioles of the plants that had been exposed to ethylene elongated less than those of the plants exposed to air only, but no quantitative study of growth was made.

Measurements of catalase activity were made in experiment 5 of this series, by the method of leaf samples already referred to. In all cases catalase activity apparently decreased during the 4-day period but, as in the other similar tests of catalase activity, ethylene treatment showed no influence on either the rapidity or the extent of this change.

PRELIMINARY EXPERIMENTS WITH WHEAT SEEDLINGS.—From the experiments thus far considered no definite nor consistent conclusions might be drawn with regard to the influence of ethylene. Satisfactory repetition of these experiments was impossible, because one could not be sure of close similarity between two lots of plant material used at different times, since as the season advances the physiological state of the plants must become more or less altered. While these experiments were in progress it became increasingly evident that a clear picture of the nature of any influence exerted by ethylene on plant processes is hardly to be expected from experimentation unless the plant material used, as well as the methods employed, may be specified with sufficient precision to permit subsequent repetition of the experiments, either by the original experimenter or by others. Only under such circumstances may the results of several experiments be compared in a satisfactory manner. Nor may the results of several presumably like experiments performed at different times be combined, as by averaging, unless all effective variables are known to be practically alike for all. The plant material used should have as nearly as possible the same internal characteristics at the beginning for all presumably comparable experiments, and the experimental conditions should be adequately specified in all cases and nearly alike for all experiments whose results are to be averaged. Furthermore, the material used needs to be described

sufficiently well so that the same or another experimenter may be able at a later time to ascertain whether his material is in physiological capacity practically like that used in earlier experiments, or, if it differs considerably, just how it differs. These requirements, which are the same as for any other line of physiological experimentation, are of course difficult to meet, and the best that may be done is to plan each study so as to approach meeting them as nearly as possible.

It was suggested that wheat seedlings of similar genetic constitution, grown from pure-line seed that had not deteriorated significantly since being harvested, might be as suitable as any other equally available higher-plant material. If all the seeds of the stock used were essentially alike, if they did not become physiologically altered in storage during the progress of the experimentation, and if they were allowed to germinate and the resulting seedlings brought to a specified stage of development under specified standard conditions, then all the seedlings thus secured should be nearly alike in physiological capacity. Such variation as occurred among these "standard" seedlings might be cared for by the employment of a sufficiently large number of seedlings in each test, so that the several lots used in simultaneous or successive tests would be much more nearly alike statistically than the individual seedlings in any lot. To test these possibilities some preliminary experiments with wheat seedlings were performed at State College, in the summer of 1928. Nittany wheat, a pure-line variety bred by Dr. C. F. NOLL, of the Pennsylvania Agricultural Experiment Station, was used, of the crop of 1927.

In the first of these experiments 200 seeds were submerged in 450 ml. of SHIVE'S (48) R_5C_2 nutrient solution in each of two wide-mouthed pint glass jars. Each liter of solution contained 0.1228 gm. of $Ca(NO_3)_2$, 0.2452 gm. of KH_2PO_4 , and 0.3698 gm. of $MgSO_4$, and the osmotic value of the solution was approximately 0.175 atm. at 20°. Each culture jar was at once inclosed in one of the bell-jar chambers used in the celery experiments just described. The inlet tube of the chamber was extended so that it led to the bottom of the nutrient solution in the culture jar but the inner end of the outlet tube remained as before. The continuous current of gas was thus caused to bubble through the nutrient solution. A mixture of one volume of ethylene in 1000 volumes of air was passed through one culture from the be-

ginning, while air only was similarly passed through the other culture. The experiment was continued for 5 days, with a maintained temperature about 25°. The rate of CO₂ production in each culture was measured at intervals of about 12 hr., by absorption and titration, and the average amounts of elongation of shoots and of roots were ascertained for the 5-day period.

Carbon-dioxide production in the culture with ethylene was somewhat less rapid at first than that in the control culture; in the interval from the 20th to the 35th hour of the experiment the two rates were practically alike; and then the ethylene-treated seedlings were again less active than the others. Consequently it appears that the influence of the maintained ethylene pressure (0.1% in air) was generally to retard CO₂ production, but that this influence was somehow overcome for the first half of the second day of the experiment. This exceptional interval occurred before the seed coats were ruptured, in the latter part of the phase of swelling of the seeds. Of course the rate of CO₂ production was very low at first in both cultures and it increased with time, but its acceleration was less, on the whole, in the presence of ethylene than in its absence. For the entire period of 5 days, elongation of both shoots and roots was markedly retarded by the ethylene treatment. At the end of the experiment the average lengths of shoots and of roots were as follows:

	AVERAGE SHOOT LENGTH	AVERAGE ROOT LENGTH
Control culture.	10.8 mm.	39.2 mm.
Culture with ethylene.	3.2	10.2

The second of these experiments with wheat seedlings began with seedlings instead of dry seeds. These had been grown in indoor daylight, at a temperature of about 25°, from seeds on mosquito netting stretched across the top of an earthenware crock full of tap water. The shoots were about 60 mm. long, and the first leaf was partly extended and showed green color. There were 50 seedlings in each of two cultures, selected by pairs that were matched in size and general appearance, so that the two lots were of very nearly the same composition, so far as might be judged by appearance. These were treated as in the preceding experiment, except that the total concentration of the nutrient solution used in this instance was 10 times as

great as that of the solution used in the first. The rates of CO_2 production for untreated and ethylene-treated seedlings were nearly alike throughout the 5-day period. The rate of shoot elongation in the treated culture was lower than in the other during the first 2 days, for which period the average increments were 43.2 mm. (treated seedlings) and 54.1 mm. (untreated seedlings). After the first 2 days the rates of shoot elongation were nearly alike in both cultures but the shoots of the treated seedlings showed more bending than the others. At the end of 4 days the untreated seedlings were somewhat greener and more uniform in size than were the treated seedlings.

The third and fourth experiments in this preliminary series were similar to the first, but each culture began with 100 dry seeds, selected for uniformity in size and appearance. Temperature fluctuated irregularly between 25° and 26° , and the length of the whole experimental period was 4 days. The ethylene concentration used was the same as before, 0.1% in air by volume. Measurements of CO_2 produced with and without ethylene in the gas stream were made at intervals of about 12 hr. Ethylene retarded CO_2 production excepting for the initial 2-day period of swelling and bursting of the seeds, in which period there was no considerable difference in CO_2 production between the treated culture and the untreated one. The retardation in CO_2 rate became progressively more pronounced throughout the remaining 2 days of the period, and finally amounted to about 25%. No growth measurements were made in these two experiments but it was evident that shoot elongation was also retarded by ethylene.

Measurements of catalase and oxidase activity were made at the end of the fourth experiment, by means of 5-seedling samples. Three samples were taken from each of the six cultures. Sample no. 1b consisted of the five seedlings with longest shoots in the culture with ethylene, and the corresponding sample from the control culture (no. 1a) consisted of five seedlings selected for size to match those in no. 1b. The second sample from the ethylene-treated culture (no. 2b) consisted of somewhat smaller seedlings and these were matched as nearly as possible in the second sample from the untreated culture (no. 2a). The third samples (nos. 3b and 3a) were chosen to represent the average of the seedling population in each case. The seed-

lings of each sample were first freed of superficial moisture by light pressing between absorbent paper, after which the weight of the sample was ascertained. The seedlings of each sample (including the remaining endosperm) were triturated with their weight of powdered CaCO_3 and 50 times their weight of distilled water, and the preparation was then allowed to stand for 24 hr. at a temperature of $3^\circ\text{--}4^\circ$.

Relative catalase activity was estimated for each preparation by the method used in the catalase experiments with celery. Relative oxidase activity was measured with a simplified Bunzel's apparatus, as described by HARVEY (21). After thorough stirring of the prepared sample, 5 ml. of the mixture were placed in one arm of the Y-shaped reaction tube and 1 ml. of a solution containing 1 gm. of pyrogallol in 100 ml. of water was introduced into the other arm. After the tube had been closed the two solutions were mixed and agitated continuously by means of a mechanical shaker making about 120 thrusts a minute, the manometer being read at 15-min. intervals for 1 hr. Oxidase activity was indicated by reduction of gas pressure inside the reaction tube, due to oxygen absorption by the pyrogallol under the influence of the oxidizing enzymes. All six tests were made simultaneously. The temperature of the tube and its contents was 27.5° .

The results of these measurements on catalase and oxidase are presented in table I. The index of relative catalase activity is of course the reciprocal of the time required for the release of a unit of oxygen, and larger numbers of seconds consequently represent lower degrees of catalase activity. On the other hand, the pressure decrements given are themselves taken as indices of oxidase activity. Throughout the table the values from ethylene-treated seedlings are in bold-face type.

The first pair of samples (1a, 1b) showed greater catalase activity and less oxidase activity for the ethylene-treated seedlings than for the untreated ones, but the converse is true for the remaining two pairs of samples. It is clear that there was much variability among the control seedlings with respect to these estimates of enzym activity, and it seems that the influence of ethylene probably differed according to the vigor of the seedlings. Ethylene apparently *increased* catalase activity and *decreased* oxidase activity in the more vigorous

seedlings but it appears to have *decreased* catalase activity and *increased* oxidase activity in somewhat less vigorous seedlings. This remarkable discrepancy suggests that serious difficulties of variability might need to be overcome if a consistent study of these physiological activities were to be undertaken by means of experiments of this sort. About all that may be said in this particular connection is that ethylene might be expected either to accelerate or to retard

TABLE I

CATALASE AND OXIDASE ACTIVITY OF WHEAT SEEDLINGS 4 DAYS AFTER DRY SEEDS HAD BEEN PLACED UNDER NUTRIENT SOLUTION THROUGH WHICH AIR OR ETHYLENE-AIR MIXTURE (1:1000) BUBBLED CONTINUALLY; TEMPERATURE ABOUT 25°

SAMPLE NO.	TREATMENT	WEIGHT OF SAMPLE (5 SEEDLINGS) (MG.)	CATALASE ACTIVITY; TIME (IN SECONDS) REQUIRED TO RELEASE		OXIDASE ACTIVITY; MANOMETRIC PRESSURE REDUCTION AFTER CONSECUTIVE 15-MIN. INTERVALS, IN MM. OF Hg			
			1 ML. OF OXYGEN	2 ML. OF OXYGEN	1ST INTER-VAL	2ND INTER-VAL	3RD INTER-VAL	4TH INTER-VAL
1a....	Control (air only)	512	82	433	4.5	14.0	23.0	31.0
1b....	Air and ethylene	485	55	230	3.5	12.4	20.8	29.5
2a....	Control (air only)	442	128	880	4.1	12.2	20.0	26.4
2b....	Air and ethylene	464	384	1590	7.1	16.0	23.0	30.2
3a....	Control (air only)	526	35	75	2.5	10.0	17.6	23.5
3b....	Air and ethylene	452	265	1345	5.4	15.5	27.5	33.3

these enzymatic processes, or to exert no influence upon them, and that if one of the processes were accelerated the other would presumably be retarded.

Main series of experiments with wheat seedlings

EXPERIMENTAL PROCEDURE

The results of the preliminary experiments already described strengthened our conviction that ethylene influence on plant processes may vary in direction as well as in amount, not only with reference to ethylene treatment and according to the kind of plant material considered, its current developmental phase and physiological

vigor, but also with reference to the prevailing complex of non-ethylene conditions. Since wheat seedlings appeared to be promising material for a study of some kinds of ethylene effects as influenced by conditions other than those dependent on ethylene itself, a consistently arranged series of experiments with wheat seedlings was planned and carried out, at the Laboratory of Plant Physiology of the Johns Hopkins University, in 1928-29. An attempt was made to take into account in a quantitative way some of the most readily suggested conditions that might influence the direction and extent of ethylene effects. The partial pressures of oxygen, of carbon dioxide and other volatile metabolic products, and of ethylene were all approximately maintained in the seedling environment for each experiment, through the employment of continuously flowing gas mixtures without CO_2 and with known contents of oxygen, nitrogen, and ethylene. A greatly superfluous supply of moisture to the seedlings was maintained by having the latter submerged in a relatively large volume of a standard nutrient solution, which also gave assurance that the supplies of mineral nutrients were initially alike for all experiments and did not alter very rapidly during the relatively short experimental period. Temperature was maintained for each experiment and light was excluded. A standard procedure was developed, so that experiments might be repeated and the results of any experiment might be comparable with those of similar or partially similar experiments performed at other times. Temperature, time, the partial pressure of oxygen and the presence or absence of ethylene at a single specified partial pressure were the experimental variables, and their effects were studied throughout an extensive series of combinations.

The experiments without ethylene were all performed first, constituting in themselves a consistent study of the influence of oxygen pressure, temperature, and time on CO_2 production and shoot elongation under the standard background conditions. The main results of that experiment series have already been reported by MACK (32). The experiments with ethylene were performed after those without ethylene had been completed, and the earlier series are of course the respective controls for the later series. The experimental technique employed was the same for both series, excepting such modifications

as were necessitated by the introduction of ethylene into the environmental complex or by limitations of time for the study as a whole. This technique and our computation procedures have been mostly described in detail by MACK (32), to whose paper reference should be made in connection with critical study of the present report.

Five different maintained temperatures (10° , 15° , 20° , 25° , 30°) were employed in each series. There were twelve different gas mixtures without ethylene, consisting of nitrogen and oxygen, with oxygen contents of 0.6, 3.1, 6.3, 9.8, 16.0, 20.0, 30.0, 50.0, 75.0, 90.0, 95.0, and 98.3% by volume. For the experiments with ethylene the nitrogen-oxygen mixtures containing 3.1, 90.0, 95.0, and 98.3% of oxygen were omitted and percentages of 16.6 and 21.0 were employed instead of 16.0 and 20.0. These last differences will be ignored in the following presentation and it will be understood that 16 and 20% mean 16.6 and 21% when reference is had to the ethylene series. To each of these eight nitrogen-oxygen mixtures ethylene was added to make up 0.1% of the total gas volume. In every experiment the gas mixture bubbled continuously through the nutrient solution in which the seedlings were submerged, renewing the oxygen supply and removing CO_2 and any other volatile products of metabolism that may have been eliminated. The total gas pressure above the culture solution was very little above that of the ordinary atmosphere, fluctuating slightly with weather changes. The rate of gas flow was about 20 ml. per minute, which had been shown, in special tests, to be more than sufficient for maximal CO_2 production and growth, so far as oxygen supply was concerned, for all the temperature-oxygen combinations tested without ethylene. The CO_2 brought away by the gas stream was collected in $\text{Ba}(\text{OH})_2$ solution and measured by titration at the same specified intervals in all experiments of both series; namely, 4, 10, 22, 34, and 46 hr. after the end of a preliminary 2-hr. interval for transfer and adjustment. The five observation intervals for measurement of CO_2 were therefore 4, 6, 12, 12, and 12 hr. long, respectively, and there were 300 different combinations of time, temperature, and partial oxygen pressure without ethylene, and 200 different combinations of the same variables with ethylene.

The standard culture solution used for all experiments in both se-

ries was SHIVE's solution R₅C₂ (48), calculated to have an osmotic value of 1.75 atm. at 20°. Each liter contained 0.1228 gm. of Ca(NO₃)₂, 0.2452 gm. of KH₂PO₄, and 0.3698 gm. of MgSO₄; no iron was added. The distilled water used was from a Barnstead still. All cultures were in 300-ml. Erlenmeyer flasks of Pyrex chemically-resistant glass, each with 250 ml. of solution and 100 standard seedlings. The standard solution must have contained very small amounts of other substances than are here mentioned, owing to inevitable impurities in the water and salts used and slight solubility of the glass containers. Some changes doubtless occurred in the essential constitution of the solution during the 48-hr. experimental period, notably because of absorption and excretion by the seedlings, but it may be tentatively supposed that such changes did not differ enough from experiment to experiment to produce significant difference in the results. Of course our experimental technique would have approached logical perfection more nearly if the culture solutions had been continuously renewed, which is highly desirable in all solution-culture experiments, as has been emphasized by TRELEASE and LIVINGSTON (55), SHIVE and STAHL (49), and PIRSCHLE (36). To develop methods and procedures for continuous solution flow that might be applied while the other requirements of these experiments were satisfied, however, would have required more time than was at our disposal. In several special tests, solutions that had previously been used as in the regular experiments, but for only 22 hr., gave somewhat more rapid CO₂ production in the first 6 hr. than was given by corresponding fresh solutions, but that difference was not evident in subsequent intervals. The question thus raised is worthy of special study.

Five cultures with different temperatures but all with gas mixture from the same tank were carried out at one time. The flasks were prepared beforehand, being charged with nutrient solution and allowed to attain the desired temperature in their respective temperature chambers before the introduction of the seedlings.

The seedlings used were all grown from the same stock of Nittany wheat as had been used for the preliminary experiments with wheat seedlings. For each set of five cultures, 1000 selected seeds were introduced into 500 ml. of distilled water in a 600-ml. flask. These

were allowed to swell and germinate for a germination period of 42 hr. at a temperature of 19.5° , during which time air was bubbled through the liquid at a rate of about 30 liters per hour, rapidly enough to produce a vigorous stirring of the water and some movement of the seeds. Nearly all of the seeds germinated with this treatment. (For a study of the influence of rate of air flow in such cultures, see TANG'S (54) recent paper.) At the end of the germination period, when coleoptiles had burst forth and roots were about to emerge from the coleorhizas, 100 selected seedlings were transferred to nutrient solution in each of the five prepared culture flasks, which were immediately returned to their respective temperature chambers; the flow of the requisite gas mixture was then started. The first interval for measuring CO_2 production began 44 hr. after the dry seeds had been placed in water for germination.

No attempt was made to avoid possible development of microorganisms, either in the germination cultures or in the regular experiments. No fungus was observed, but there was doubtless considerable development of bacteria, and perhaps of other microorganisms, on the seeds and seedlings and in the culture medium, for, as has been said, the same charge of liquid was used throughout the experimental period without renewal. If the influence of microorganisms on the rate of CO_2 production or on the rate of shoot elongation was significant, it is embraced in the general background complex for all these experiments. But the possibility of such influence should naturally be borne in mind in connection with the interpretation of the results of such studies as this. It should be explained, perhaps, that this study deals not just with the activities of wheat seedlings as living material but with their activities along with the concomitant activities of any and all microorganisms that may have been present in the cultures. Our discussion proceeds, however, as though microorganisms were known to have been uninfluential with respect to the process rates considered.

The experiments with ethylene differed essentially from their respective controls (the corresponding ones without ethylene) only in that their flowing gas mixtures all contained 0.1% of ethylene by volume. Ethylene was measured into the mixing cylinder before either oxygen or nitrogen was introduced, so that its pressure could

be measured with a mercury manometer instead of with the less accurate pressure gauge used for the much greater pressures of oxygen and nitrogen. The ethylene used was of the grade commonly employed for anaesthesia, and was obtained from the Kansas City Oxygen Gas Company. Ethylene was regularly admitted into the mixing cylinder until the pressure therein was 1 pound (5.1 cm. on the manometer scale); oxygen and nitrogen were then admitted in the requisite proportions, making up a total pressure of 1000 pounds, read on the pressure gauge. The oxygen percentage of each gas mixture was ascertained, for a sample, by absorption of oxygen with phosphorus (as was also done in the case of the controls), and this percentage was corrected, if necessary, by adding small amounts of oxygen or nitrogen until sample tests showed that the desired percentage had been very nearly attained.

Only one ethylene concentration was tested. This concentration (0.1% by volume) is the same as was used in the preliminary experiments, described in the earlier part of this paper. As has been noted, it has been recommended for blanching celery, ripening tomatoes, etc. It has been reported, for example, by WALLACE (59) and DENNY (7), that the nature of the effects of ethylene was qualitatively the same for a wide range of concentrations, although it might differ quantitatively for relatively small differences in concentration. It is obvious, however, that much further experimentation will be required when the concentration of ethylene comes to be studied as an experimental variable in this sort of investigation.

At the end of the fifth interval of each experiment (48 hr. after the standard seedlings had been placed in the experimental environment), the average shoot length of the longest ten seedlings in each culture was ascertained and this average was taken to represent the growth activity of the plantlets for the whole experimental period.

NUMERICAL DATA

The primary results of this study comprise (1) the mean hourly rates of CO_2 production by 100 seedlings for each of the five observation intervals, and (2) the growth indices secured at the end of the experimental period. These values were simply averaged for each group of two or more like experiments and the resulting final averages are shown in tables II-VI. The values from the separate experi-

TABLE II
AVERAGE HOURLY RATES OF CO₂ PRODUCTION IN 1ST AND 2ND INTERVALS,
WITH CORRESPONDING ETHYLENE EFFECTS

OXYGEN PRESSURE (%)	TEMPERA- TURE (° C.)	FIRST INTERVAL (3RD TO 6TH HR. INCLUSIVE)			SECOND INTERVAL (7TH TO 12TH HR. INCLUSIVE)		
		WITHOUT ETHYLENE (MG.)	WITH ETHYLENE, 0.1% (MG.)	ETHYLENE EFFECT (%)	WITHOUT ETHYLENE (MG.)	WITH ETHYLENE, 0.1% (MG.)	ETHYLENE EFFECT (%)
0.6	10	0.36	0.34	- 6	0.26	0.25	- 4
	15	0.46	0.46	0	0.41	0.39	- 5
	20	0.63	0.63	0	0.61	0.61	0
	25	0.74	0.81	+10	0.79	0.78	- 1
	30	0.96	0.97	+ 1	1.11	0.96	-13
6.3	10	0.38	0.38	0	0.31	0.31	0
	15	0.61	0.58	- 5	0.56	0.59	+ 5
	20	0.73	0.67	- 8	0.77	0.76	- 1
	25	0.93	0.89	- 4	1.14	1.08	- 5
	30	1.16	1.23	+ 6	1.72	1.73	+ 1
9.8	10	0.42	0.42	0	0.34	0.37	+ 9
	15	0.56	0.63	+12	0.54	0.62	+15
	20	0.86	0.81	- 6	0.80	0.88	+10
	25	1.02	1.03	+ 1	1.37	1.38	+ 1
	30	1.21	1.32	+ 9	1.86	1.99	+ 7
16.0	10	0.44	0.41	- 7	0.41	0.41	0
	15	0.58	0.60	+ 3	0.56	0.55	- 2
	20	0.76	0.80	+ 5	0.83	0.92	+11
	25	0.97	1.12	+16	1.20	1.44	+20
	30	1.23	1.32	+ 7	1.75	1.97	+13
20.0	10	0.39	0.39	0	0.41	0.40	- 2
	15	0.63	0.59	- 8	0.64	0.65	+ 2
	20	0.82	0.80	- 2	0.84	0.91	+ 8
	25	0.95	0.94	- 1	1.16	1.21	+ 4
	30	1.30	1.35	+ 4	1.76	1.75	- 1
30.0	10	0.44	0.50	+14	0.54	0.56	+ 4
	15	0.71	0.72	+ 1	0.72	0.73	+ 1
	20	0.83	0.95	+14	0.94	1.13	+20
	25	1.05	1.12	+ 7	1.24	1.46	+18
	30	1.34	1.45	+ 8	1.69	1.92	+14
50.0	10	0.45	0.51	+13	0.56	0.54	- 4
	15	0.74	0.72	- 3	0.85	0.83	- 2
	20	0.94	0.90	- 4	1.05	1.07	+ 2
	25	1.20	1.20	0	1.44	1.52	+ 6
	30	1.60	1.56	- 3	2.02	1.90	- 6
75.0	10	0.47	0.56	+19	0.59	0.58	- 2
	15	0.82	0.77	- 6	0.98	1.01	+ 3
	20	1.10	0.96	-13	1.37	1.31	- 4
	25	1.38	1.38	0	1.81	1.95	+ 8
	30	1.91	1.90	- 1	2.56	2.66	+ 4

ments also are given, excepting those for CO_2 production in the 1st and 2nd intervals (table II), for it seems desirable to show the kind and extent of the deviation or variability encountered; they are omitted for CO_2 production in the first two intervals to conserve space and because our discussion of CO_2 production will deal mainly with the data for the last three intervals. As was suggested by MACK (32), the 2-hr. interval for adjustment (at the beginning of each experiment) may perhaps have been somewhat too short a period; there may have been some after-effects of previous culture or of transfer still more or less prevalent after the beginning of the first observation interval. The seedlings were of course larger and more active in the later intervals and the actual amounts of CO_2 measured were correspondingly larger, with relatively smaller errors of titration. Furthermore, the last three intervals were of the same length (12 hr.), while the 1st and 2nd intervals were shorter and of unequal length (4 and 6 hr. respectively), and the CO_2 rates for the last three intervals are somewhat more clearly consistent in their own group and in the whole series of five than are the corresponding data for the first two intervals. Some of the values given in these tables have previously been published by MACK (32).

Only the final averages will be considered. Each is derived from two or more like tests, as is seen from tables III-VI, but the number of duplicates is generally far too small to warrant any attempt to apply statistical methods, even if that might be deemed desirable in studies of this sort. The degree of significance of the final averages is to be appraised in terms of their consistency, as will appear from the following discussions.

Because the CO_2 data for the last three intervals are remarkably consistent, they were combined to give a single series to represent those three intervals taken together, and the resulting average CO_2 rates are set forth in table VII. They will be used in comparing CO_2 production with shoot elongation. As has been said, the indices of shoot elongation are all based on measurements made at the end of the whole experimental period, and they really represent the germination and experimental periods combined. However, the shoots of the standard seedlings were never more than 1 mm. long when brought under the experimental conditions.

Each of the final average values for CO₂ production without ethylene (control values) was subtracted from the corresponding value for ethylene treatment and the difference divided by the control

TABLE III
DETAILED DATA ON CO₂ PRODUCTION IN 3RD INTERVAL
(13TH TO 24TH HR. INCLUSIVE)

OXY- GEN PRES- SURE (%)	TEM- PERA- TURE (° C.)	HOURLY RATES OF CO ₂ PRODUCTION (MG.)				ETHY- LENE EFFECT (%)	
		WITHOUT ETHYLENE		WITH ETHYLENE, 0.1 %			
		SEPARATE TESTS		AVE.	SEPARATE TESTS		AVE.
0.6	10	0.28, 0.27, 0.27	0.27	0.26, 0.25	0.26	- 4	
	15	0.45, 0.43, 0.50	0.46	0.46, 0.48	0.47	+ 2	
	20	0.66, 0.64, 0.68	0.66	0.67, 0.67	0.67	+ 2	
	25	1.01, 0.95, 0.83	0.93	0.88, 0.94	0.91	- 2	
	30	1.43, 1.57, 1.11	1.37	1.25, 1.35	1.30	- 5	
6.3	10	0.36, 0.32	0.34	0.33, 0.31	0.32	- 6	
	15	0.57, 0.64	0.61	0.71, 0.71	0.71	+16	
	20	1.08, 1.13	1.11	0.91, 1.18	1.05	- 5	
	25	1.93, 1.68	1.81	1.67, 1.73	1.70	- 6	
	30	2.55, 2.02	2.29	2.13, 2.36	2.25	- 2	
9.8	10	0.34, 0.32	0.33	0.39, 0.36, 0.29	0.35	+ 6	
	15	0.51, 0.52	0.52	0.70, 0.60, 0.78	0.69	+33	
	20	1.07, 1.05	1.06	1.30, 1.10, 1.27	1.22	+15	
	25	1.88, 2.02	1.95	2.16, 2.40, 2.02	2.19	+12	
	30	2.37, 2.34	2.36	2.62, 2.96, 2.80	2.79	+18	
16.0	10	0.43, 0.34, 0.43, 0.49	0.44	0.48, 0.46, 0.48	0.47	+ 7	
	15	0.62, 0.63, 0.59, 0.63	0.62	0.67, 0.76, 0.72	0.72	+16	
	20	1.11, 1.07, 0.97, 1.18	1.08	1.30, 1.17, 1.15	1.21	+12	
	25	1.89, 1.47, 1.71, 1.88	1.74	2.01, 1.84, 1.85	1.90	+ 9	
	30	2.62, 2.54, 2.70	2.62	2.29, 2.75, 2.66	2.57	- 2	
20.0	10	0.36, 0.37, 0.52, 0.51, 0.45, 0.48, 0.47	0.45	0.44, 0.38	0.41	- 9	
	15	0.77, 0.76, 0.83, 0.84, 0.73, 0.71, 0.73	0.77	0.77, 0.82	0.80	+ 4	
	20	1.07, 1.03, 1.09, 1.06, 1.02, 1.04, 1.10	1.07	1.14, 1.12	1.13	+ 6	
	25	1.37, 1.35, 1.59, 1.42, 1.61, 1.40, 1.47	1.46	1.33, 1.40	1.40	- 4	
	30	2.13, 2.38, 2.47, 2.37, 2.32, 2.14, 2.14	2.28	2.17, 2.13	2.15	- 4	
30.0	10	0.58, 0.59, 0.60	0.59	0.66, 0.55	0.61	+ 3	
	15	0.87, 0.80, 0.83	0.83	0.89, 0.92	0.91	+10	
	20	1.10, 1.25, 1.27	1.21	1.32, 1.34	1.33	+10	
	25	1.61, 1.73, 1.66	1.67	1.78, 1.78	1.78	+ 7	
	30	2.07, 2.41, 2.28	2.25	2.43, 2.48	2.46	+ 9	
50.0	10	0.67, 0.71	0.69	0.63, 0.58	0.61	-12	
	15	0.90, 0.98	0.97	1.03, 0.93	0.98	+ 1	
	20	1.31, 1.54	1.43	1.38, 1.31	1.35	- 6	
	25	1.79, 2.07	1.93	2.02, 1.94	1.98	+ 3	
	30	2.04, 2.82	2.88	2.46, 2.68	2.57	-11	
75.0	10	0.77, 0.78, 0.73, 0.73	0.75	0.75, 0.66	0.71	- 5	
	15	1.16, 1.37, 1.25, 1.42	1.30	1.33, 1.13	1.23	- 8	
	20	2.05, 1.87, 1.92, 2.08	1.98	2.04, 1.62	1.83	- 8	
	25	2.65, 2.78, 2.70, 3.05	2.80	3.19, 2.50	2.85	+ 2	
	30	3.92, 3.68, 4.04	3.88	3.96, 3.62	3.79	- 2	

value. The quotient was finally multiplied by 100, to give an index of ethylene effect expressed as a percentage based on the corresponding control value. This index may be: (1) positive (when the aver-

age rate is greater with ethylene than without it; that is, when ethylene apparently accelerated CO_2 production); (2) zero (when ethylene treatment showed no effect); or (3) negative (when the

TABLE IV
DETAILED DATA ON CO_2 PRODUCTION IN 4TH INTERVAL
(25TH TO 36TH HR. INCLUSIVE)

OXY- GEN PRES- SURE (%)	TEM- PERA- TURE (° C.)	HOURLY RATES OF CO ₂ PRODUCTION (MG.)				ETHYL- ENE EFFECT (%)
		WITHOUT ETHYLENE		WITH ETHYLENE, C.I. %		
		SEPARATE TESTS	AVE.	SEPARATE TESTS	AVE.	
0.6	10	0.29, 0.27, 0.27.....	0.28	0.32, 0.28.....	0.30	+ 7
	15	0.47, 0.45, 0.50.....	0.47	0.51, 0.49.....	0.50	+ 6
	20	0.67, 0.71, 0.72.....	0.70	0.73, 0.66.....	0.70	0
	25	1.07, 1.05, 0.96.....	1.03	1.06, 1.10.....	1.08	+ 5
	30	1.59, 1.43, 1.24.....	1.42	1.37, 1.55.....	1.46	+ 3
6.3	10	0.36, 0.38.....	0.37	0.43, 0.31.....	0.37	0
	15	0.75, 0.89.....	0.82	0.97, 0.80.....	0.93	+13
	20	1.42, 1.33.....	1.38	1.40, 1.45.....	1.43	+ 4
	25	2.28, 1.83.....	2.06	2.00, 1.82.....	1.90	- 5
	30	2.84, 2.28.....	2.56	2.00, 2.72.....	2.81	+10
9.8	10	0.34, 0.38.....	0.36	0.51, 0.44.....	0.48	+33
	15	0.67, 0.73.....	0.70	0.98, 0.77.....	0.88	+26
	20	1.19, 1.10.....	1.18	1.75, 1.36.....	1.56	+32
	25	2.38, 2.23.....	2.31	2.50, 2.58.....	2.54	+10
	30	3.23, 3.15.....	3.19	3.23, 3.40.....	3.32	+ 4
16.0	10	0.47, 0.51, 0.53, 0.53.....	0.51	0.55, 0.53, 0.55.....	0.54	+ 6
	15	0.68, 0.75, 0.84, 0.81.....	0.77	0.91, 0.93, 0.80.....	0.90	+17
	20	1.18, 1.24, 1.04, 1.22.....	1.17	1.50, 1.36, 1.22.....	1.36	+16
	25	2.20, 2.17, 2.15, 2.30.....	2.21	2.40, 2.35, 2.40.....	2.40	+ 4
	30	3.87, 3.70, 3.70.....	3.76	3.06, 3.73, 4.06.....	3.62	- 4
20.0	10	0.39, 0.47, 0.66, 0.58, 0.54, 0.54.....	0.53	0.53, 0.40.....	0.47	-11
	15	0.94, 0.93, 1.01, 0.98, 0.91, 0.88.....	0.94	0.91, 0.92.....	0.92	- 2
	20	1.18, 1.20, 1.31, 1.41, 1.20, 1.50.....	1.33	1.32, 1.18.....	1.25	- 6
	25	1.76, 1.75, 2.04, 2.07, 1.77, 1.98.....	1.90	1.60, 1.76.....	1.68	-12
	30	2.78, 3.27, 3.57, 3.49, 3.01, 3.21.....	3.22	3.06.....	3.06	- 5
30.0	10	0.68, 0.72, 0.71.....	0.70	0.75, 0.66.....	0.71	+ 1
	15	1.07, 1.02, 1.03.....	1.04	1.08, 1.14.....	1.11	+ 7
	20	1.31, 1.52, 1.54.....	1.46	1.72, 1.65.....	1.69	+16
	25	2.03, 2.07, 1.90.....	2.00	2.40, 2.27.....	2.34	+17
	30	2.84, 3.22, 3.27.....	3.11	3.60, 3.43.....	3.52	+13
50.0	10	0.82, 0.80.....	0.86	0.72, 0.73.....	0.73	-15
	15	1.26, 1.23.....	1.25	1.23, 1.22.....	1.23	- 2
	20	1.70, 1.07.....	1.88	1.76, 1.69.....	1.73	- 8
	25	2.02, 2.88.....	2.75	2.40, 2.66.....	2.53	- 8
	30	4.51, 4.75.....	4.63	3.78, 3.98.....	3.88	-16
75.0	10	0.87, 0.88, 0.77, 0.75.....	0.82	0.90, 0.73.....	0.82	0
	15	1.53, 1.76, 1.57, 1.75.....	1.66	1.79, 1.51.....	1.65	- 1
	20	2.86, 2.56, 2.82, 3.05.....	2.82	3.24, 2.47.....	2.86	+ 1
	25	3.77, 3.88, 3.04, 4.37.....	3.99	4.45.....	4.45	+12
	30	5.20, 5.00, 5.47.....	5.22	5.48, 4.80.....	5.14	- 2

presence of ethylene apparently acted to retard CO_2 production). Percentage indices of ethylene effect on shoot elongation were computed in a similar manner. In the columns so designated (tables II-

VII) these percentages of ethylene effect are shown for CO₂ production in each of the five observation intervals and in the last three intervals combined, as well as for shoot elongation.

TABLE V
DETAILED DATA ON CO₂ PRODUCTION IN 5TH INTERVAL
(37TH TO 48TH HR. INCLUSIVE)

OXY- GEN PRES- SURE (%)	TEM- PERA- TURE (° C.)	HOURLY RATES OF CO ₂ PRODUCTION (MG.)						ETHYL- ENE EFFECT (%)
		WITHOUT ETHYLENE			WITH ETHYLENE, 0.1%			
		SEPARATE TESTS		Ave.	SEPARATE TESTS		Ave.	
0.6	10	0.31, 0.27, 0.33.....	0.30	0.32, 0.27.....	0.30	0		
	15	0.52, 0.50, 0.49.....	0.50	0.50, 0.50.....	0.50	0		
	20	0.76, 0.83, 0.80.....	0.80	0.79, 0.75.....	0.77	-4		
	25	1.32, 1.25, 1.12.....	1.23	1.13, 1.14.....	1.14	-7		
	30	1.50, 1.54, 1.30.....	1.48	1.48, 1.72.....	1.60	+8		
6.3	10	0.42, 0.47.....	0.45	0.46, 0.46.....	0.46	+2		
	15	1.00, 1.03.....	1.02	1.26, 1.20.....	1.23	+21		
	20	1.50, 1.58.....	1.54	1.73, 1.61.....	1.67	+8		
	25	2.30, 2.30.....	2.30	2.52, 2.22.....	2.37	+3		
	30	3.36, 2.76.....	3.06	3.67, 3.58.....	3.63	+19		
9.8	10	0.40, 0.38.....	0.39	0.53, 0.52.....	0.53	+16		
	15	0.87.....	0.87	1.17, 1.07.....	1.12	+20		
	20	1.53, 1.57.....	1.55	1.91, 1.80.....	1.90	+23		
	25	3.09, 2.76.....	2.93	2.91, 2.98.....	2.95	+1		
	30	3.82, 3.61.....	3.72	3.54, 4.16.....	3.85	+3		
16.0	10	0.55, 0.56, 0.62, 0.65.....	0.60	0.62, 0.61.....	0.62	+3		
	15	0.76, 0.87, 1.03, 0.90.....	0.91	1.08, 1.04, 1.04.....	1.05	+15		
	20	1.42, 1.34, 1.42, 1.53.....	1.43	1.81, 1.73, 1.54.....	1.69	+18		
	25	2.74, 2.75, 3.32, 3.07.....	2.97	3.28, 3.19, 2.92.....	3.13	+5		
	30	5.03, 4.33, 4.63.....	4.66	3.89, 4.20, 4.55.....	4.21	+10		
20.0	10	0.46, 0.49, 0.73, 0.68, 0.63, 0.68.....	0.61	0.56, 0.60.....	0.58	-5		
	15	1.12, 1.08, 1.19, 1.22, 1.22, 1.00.....	1.15	1.07, 1.22.....	1.15	0		
	20	1.50, 1.69, 1.58, 1.69, 1.71, 1.87.....	1.67	1.63, 1.43.....	1.53	-8		
	25	2.26, 2.34, 2.72, 2.49, 2.33, 2.51.....	2.46	2.06, 2.58.....	2.32	-6		
	30	3.63, 3.82, 4.50, 4.03, 4.00.....	4.00	3.92, 4.52.....	4.22	+6		
30.0	10	0.81, 0.84, 0.83.....	0.83	0.81, 0.81.....	0.81	-2		
	15	1.35, 1.33, 1.33.....	1.34	1.40, 1.38.....	1.39	+4		
	20	1.71, 1.84, 1.05.....	1.83	2.33, 2.15.....	2.24	+22		
	25	2.80, 2.77, 2.68.....	2.75	3.36, 3.95.....	3.21	+17		
	30	4.10, 4.59, 4.43.....	4.34	4.68, 4.38.....	4.53	+4		
50.0	10	1.02, 1.07.....	1.05	0.86, 0.88.....	0.87	-17		
	15	1.66, 1.75.....	1.71	1.61, 1.50.....	1.56	-9		
	20	2.44, 2.72.....	2.58	2.20, 2.28.....	2.24	-13		
	25	3.50, 4.24.....	3.87	3.33, 3.50.....	3.42	-12		
	30	5.67, 5.95.....	5.81	4.61, 5.32.....	4.97	-14		
75.0	10	1.08, 1.17, 0.97, 1.00.....	1.06	1.16, 0.86.....	1.01	-5		
	15	2.34, 2.41, 2.10, 2.50.....	2.41	2.42, 2.06.....	2.24	-7		
	20	3.81, 3.66, 3.95, 3.83.....	3.81	4.26, 3.70.....	3.98	+4		
	25	4.42, 5.17, 4.83, 5.32.....	4.94	5.65, 5.04.....	5.35	+8		
	30	6.06, 5.99, 6.36.....	6.14	5.66, 5.97.....	5.82	-5		

Those tests without ethylene for which there were no corresponding tests with ethylene are not generally considered in this paper and the data for them do not appear in the tables. They were with oxy-

gen pressures of 3.1, 90.0, 95.0, and 98.3%. Because of lack of time for further experimentation ethylene treatment with these oxygen pressures was not tested. The results of the tests without ethylene,

TABLE VI
DETAILED DATA ON SHOOT ELONGATION (WHOLE PERIOD)

OXY- GEN PRES- SURE (%)	TEM- PERA- TURE (° C.)	AVERAGE LENGTHS OF LONGEST 10 SHOOTS (MM.)				ETHYL- ENE EFFECT (%)
		WITHOUT ETHYLENE		WITH ETHYLENE, 0.1%		
		SEPARATE TESTS	AVE.	SEPARATE TESTS	AVE.	
6.3	10	1.7, 1.4.....	1.6	0, 0.....	0	-100
	15*	1.7, 1.4.....	1.6	0, 0.....	0	-100
9.8	10	2.4, 2.3.....	2.4	1.6, 1.6.....	1.6	-33
	15	2.9, 2.7.....	2.8	1.8, 2.1.....	2.0	-29
	20*	2.6, 2.6.....	2.6	0, 0.....	0	-100
16.0	10	2.6, 2.6, 3.2, 2.9.....	2.8	2.5, 2.7, 2.3.....	2.5	-11
	15	3.5, 3.3, 4.0, 4.2.....	3.8	3.5, 3.6, 3.4.....	3.5	-8
	20	3.0, 3.2, 2.5, 3.4.....	3.0	3.5, 3.9, 3.4.....	3.6	+20
	25*	2.5, 2.5, 2.3, 2.4.....	2.4	2.3, 2.2, 2.0.....	2.2	-8
20.0	10	3.0, 2.7, 2.7, 3.6, 3.4, 2.7, 2.7.....	3.0	1.2, 2.2.....	2.0	-33
	15	5.4, 4.1, 4.7, 6.3, 5.9, 3.8, 3.7.....	4.8	3.3, 3.5.....	3.4	-49
	20	5.8, 7.1, 8.8, 7.0, 9.3, 6.4, 5.6.....	7.2	3.9, 4.4.....	4.2	-42
	25	4.8, 8.2, 11.2, 5.4, 10.2, 6.0, 4.8.....	7.2	4.5, 4.9.....	4.7	-35
	30	2.3, 2.2, 2.5, 2.0, 2.5, 1.9, 2.0.....	2.2	0, 0.....	0	-100
30.0	10	2.7, 3.4, 2.9.....	3.0	2.6, 2.5.....	2.6	-13
	15	5.0, 4.7, 5.4.....	5.0	4.7, 3.7.....	4.2	-16
	20	10.4, 6.9, 10.3.....	9.2	6.6, 5.3.....	5.9	-36
	25	7.4, 5.7, 10.3.....	7.8	6.1, 5.1.....	5.6	-28
	30	3.1, 3.1, 4.1.....	3.4	2.8, 3.0.....	2.9	-15
50.0	10	3.0, 3.0.....	3.0	2.5, 2.4.....	2.5	-17
	15	5.4, 5.4.....	5.4	3.9, 4.3.....	4.1	-24
	20	10.2, 7.8.....	9.0	4.7, 6.0.....	5.4	-40
	25	11.4, 10.6.....	11.0	5.9, 5.0.....	5.3	-52
75.0	10	2.8, 4.7.....	3.8	4.4, 4.2.....	4.3	+13
	15	2.2, 2.4, 2.3, 2.5.....	2.4	2.2, 1.8.....	2.0	-17
	20	3.0, 5.1, 4.0, 3.9.....	4.0	4.5, 4.4.....	4.5	+13
	25	6.1, 7.6, 7.6, 5.0.....	6.6	7.3, 7.9.....	7.6	+15
95.0	15	7.8, 9.7, 8.0, 6.6.....	8.0	7.3, 7.5.....	7.4	-8
	30	3.1, 6.9, 3.8, 3.8.....	4.4	5.9, 6.1.....	6.0	+36

* There was no measurable shoot growth, either with or without ethylene, for any tested temperature with oxygen pressure of 0.6%. The same is true for 20°, 25°, and 30° with oxygen pressure of 6.3%; for 25° and 30° with oxygen pressure of 9.8%; and for 30° with oxygen pressure of 16.0%. Consequently these combinations of temperature and oxygen pressure do not appear in this table.

however, are given by MACK (32), and some of them will be mentioned in our discussion.

PRIMARY GRAPHS OF CO₂ PRODUCTION

METHOD OF PLOTTING.—The final average hourly rates of CO₂ production in each of the last three observation intervals and in these three intervals taken together are shown graphically in figure 1.

TABLE VII

AVERAGE HOURLY RATES OF CO₂ PRODUCTION IN 3RD, 4TH, AND 5TH INTERVALS COMBINED (13TH TO 48TH HR.), TOGETHER WITH CORRESPONDING ETHYLENE EFFECTS ON CO₂ PRODUCTION AND ON SHOOT ELONGATION (GROWTH DATA ARE FROM TABLE VI)

OXYGEN PRESSURE (%)	TEMPERATURE (° C.)	AVERAGE HOURLY RATE OF CO ₂ PRODUCTION (MG.)		ETHYLENE EFFECT (%)	
		WITHOUT ETHYLENE	WITH ETHYLENE, 0.1%	ON CO ₂ PRODUCTION	ON SHOOT ELONGATION
0.6	10	0.28	0.29	+ 4
	15	0.48	0.49	+ 2
	20	0.72	0.71	- 2
	25	1.06	1.04	- 2
	30	1.42	1.45	+ 2
6.3	10	0.39	0.38	- 3	-100
	15	0.82	0.96	+17	-100
	20	1.34	1.38	+ 3
	25	2.06	2.01	- 2
	30	2.64	2.90	+10
9.8	10	0.36	0.45	+25	- 33
	15	0.70	0.90	+29	- 29
	20	1.26	1.56	+24	-100
	25	2.40	2.56	+ 7
	30	3.09	3.32	+ 7
16.0	10	0.52	0.54	+ 4	- 11
	15	0.77	0.89	+16	- 8
	20	1.23	1.42	+15	+ 20
	25	2.31	2.48	+ 7	- 8
	30	3.68	3.47	- 6
20.0	10	0.53	0.49	- 8	- 33
	15	0.95	0.96	+ 1	- 29
	20	1.36	1.30	- 4	- 42
	25	1.94	1.80	- 7	- 35
	30	3.17	3.14	- 1	-100
30.0	10	0.71	0.71	0	- 13
	15	1.07	1.14	+ 7	- 16
	20	1.50	1.75	+17	- 36
	25	2.14	2.44	+14	- 28
	30	3.23	3.50	+ 8	- 15
50.0	10	0.87	0.74	-15	- 17
	15	1.31	1.26	- 4	- 24
	20	1.96	1.77	-10	- 40
	25	2.85	2.64	- 7	- 52
	30	4.44	3.81	-14	+ 13
75.0	10	0.88	0.85	- 3	- 17
	15	1.79	1.71	- 4	+ 13
	20	2.87	2.80	+ 1	+ 15
	25	3.91	4.22	+ 8	- 8
	30	5.08	4.92	- 3	+ 36

Each section of the figure comprises five separate pairs of graphs, a pair for each of the five maintained temperatures tested. All are plotted on the same scales. Abscissas represent oxygen pressure in

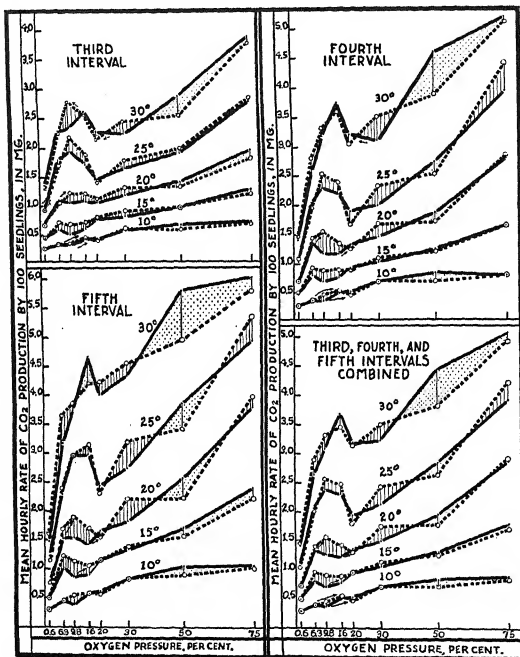


FIG. 1.—Oxygen-pressure graphs of CO_2 production in 3rd, 4th, and 5th intervals separately and combined, at five maintained temperatures, without ethylene (continuous lines) and with ethylene (broken lines). Arrows mark abscissal differences of critical points between control graphs and graphs for series with ethylene; hachured areas indicate ethylene acceleration of CO_2 production and stippled areas indicate ethylene retardation. Data are from tables III, IV, V, and VII.

the gas stream, from 0.6 to 75.0%, and ordinates represent the average hourly rates of CO_2 production. In each pair of graphs, the continuous line represents tests without ethylene (controls) and the broken line represents tests with ethylene. Positive ethylene influence (acceleration) is of course indicated where the broken line is above the corresponding continuous line (hachured areas); negative influence (retardation) is indicated where the broken line is below (stippled areas); and absence of any ethylene effect is indicated where the two graphs of a pair intersect. No smoothing has been applied to these graphs, to introduce presumable curvatures in place of straight-line segments and angles; only data that are actually available are represented. The new contribution on CO_2 production is shown by the broken-line graphs and their relations to the corresponding continuous lines.

The relations brought out by the several sets of graphs for the separate observation intervals are so nearly alike, and such differences as there are among the sets are generally so inconsistent, that it is concluded that time (or developmental phase) was apparently not an effective variable in this study. The discussion will therefore refer mainly to the data for the last three intervals combined.

MACK'S DOUBLE OPTIMUM OF OXYGEN PRESSURE FOR CO_2 PRODUCTION.—MACK (32) has already pointed out that this series of tests without ethylene showed not only the main oxygen-pressure optimum for CO_2 production (about 90–95%) but also a secondary optimal pressure with a very much lower value (between about 6.3 and about 16%, according to temperature). He has noted also that the latter value tended to be greater with higher temperature. MACK's secondary optimal pressure is of course the abscissa of the first graph maximum on any of the continuous-line graphs of figure 1. A typical illustration is the continuous-line graph for 25° and for the last three intervals combined. That graph starts, at the left, with a very low ordinate (first graph minimum) for oxygen pressure of 0.6%, ascends to the first maximal point (with abscissa of 9.8%, the secondary optimum just mentioned), reverses and descends to a second minimal point with abscissa of 20%, and then reverses again and ascends toward the primary or main graph maximum, shown by MACK's data as having an abscissa of 90–95%. The presence of the

second minimum naturally involves two critical abscissas, the ordinate for the first (first graph maximum) being greater than that for the second (second graph minimum).

The control graphs (continuous lines) of figure 1 generally agree in showing these two critical points, and their configuration for any temperature appears to be the same for all three intervals here considered, as well as for the last three intervals combined. That is true for the ethylene-treatment graph also (broken lines of figure 1), excepting those for 10° and 30° in the 5th interval. MACK's evidence for the presence of the double optimum was based on just the data from the experiment series without ethylene, and the fact that these data from the series with ethylene so consistently support his evidence in this connection renders that already convincing evidence much more convincing; for the experiment series with ethylene was carried out independently and after the other series had been completed, as has been said. Whatever the effect of ethylene may have been, its presence in the gas stream surely did not generally prevent the appearance of MACK's double optimum of oxygen pressure for CO₂ production.

The secondary optimum is of course least clearly evident on the graphs for the lowest temperature, for which all ordinates are low, and the difference in ordinate magnitude between the first graph maximum and the second graph minimum is greatest for temperatures of 25° and 30°. This difference is generally at least as great for the broken-line as for the continuous-line of the same graph pair. For both the control series and the series with ethylene, the abscissa of the first graph maximum (which is MACK's lower or secondary optimum of oxygen pressure for CO₂ production) varied between 6.3 and 16%, and there appears to be a tendency for this critical pressure to be somewhat greater with higher temperature. For 10° and 20° it is greater with ethylene than without ethylene, but for the other tested temperatures it is alike for both series. In general, the second graph minimum has an abscissa extending 4-10 percentage units beyond the corresponding abscissa of the first graph maximum. Shiftings of the abscissal positions of these two critical points, as apparent ethylene effects, are indicated in figure 1 by small arrows pointing from a maximum or minimum on a continuous-line graph toward

the corresponding critical point on the adjacent broken-line graph. With further study these shiftings may prove to be of physiological significance, but the limitations of our data make it unnecessary to attempt here any reasoned explanations of them. For the present we may be content with the general statement that MACK's secondary oxygen-pressure optimum for all tested temperatures is shown as between 6.3 and 16% for both the control tests and those with ethylene, while the second critical point (second graph minimum) is shown for all tested temperatures as having an abscissa between 9.8 and 20% for both series. The abscissal difference between these two points of inflection may be as small as 4 percentage units or as large as 10.

For the series without ethylene, as MACK has shown, the main oxygen pressure optimum is shown as 90 or 95%; this statement covers all tested temperatures. Pressure of 98.3% was clearly super-optimal for CO₂ production when ethylene was not present.

MINIMAL AND MAXIMAL OXYGEN PRESSURES AND TEMPERATURES.—

Our series of oxygen pressures and of temperatures were neither of them sufficiently extended to show any temperature-oxygen combinations preventing the elimination of CO₂. From the nature of the respiratory process, it seems safe to suppose that no minimal oxygen pressure for CO₂ production (at least with our background conditions) might be found for any temperature in either series. The initial slopes of the oxygen-pressure graphs of figure 1 all suggest pressure minima below zero, which of course refers to truly anaerobic production of CO₂. In a similar manner, it is safe to suppose that any maximal oxygen pressures that might perhaps be possible would surely be greater than 100% without ethylene, and probably also with ethylene. It will be remembered that the series without ethylene extended to a pressure of 98.4%, which is clearly superoptimal but still gave very rapid rates of CO₂ production (32). It must always remain logically impossible to test any oxygen pressure considerably above 100% without at the same time introducing a corresponding alteration in the background complex of our study, which involves a total gas pressure of about one atmosphere; the parameter limits of the investigation definitely preclude the employment of oxygen pressures higher than about one atmosphere. It is of course

logically possible, so far as our experiments go, that a maximal oxygen pressure for CO_2 production with ethylene might be found between 75 and 100%, for no oxygen pressures in that range have been tested with ethylene; but many considerations make such a supposition highly improbable.

With reference to temperature limits of CO_2 production, it is clear that the minimal temperature for appreciable maintenance of this process, either without ethylene or with it, must lie considerably below our lowest temperature (10°) for every tested oxygen pressure, probably very far below that temperature and perhaps well into the negative region of the thermometer scale. The maximal temperature for CO_2 production with any oxygen pressure, in either series of experiments, is obviously shown as above our highest tested temperature (30°), probably far above. But in this we are led, again by general considerations, to the thought (already familiar in the literature) that temperature maxima for this process are practically inconceivable; for CO_2 production is well known to continue indefinitely with higher temperature after a lethal temperature has been surpassed.

HIGHEST RATES OF CO_2 PRODUCTION.—The temperature-oxygen combinations that gave the highest rates of CO_2 production in both series of tests were those of high temperature and great oxygen pressure. Without ethylene the optimal combination is shown (32) as 30° and 95%, with a mean hourly rate (for 100 seedlings in the last three observation intervals) of 5.86 mg. The two corresponding rates nearest to this one were given by 30° combined with 90% (5.75 mg.) and with 98.3% (5.31 mg.). The combinations of 25° with 95, with 90, and with 98.3% gave the three next lower rates (4.87, 4.51, and 4.52 mg., respectively). For combinations including only oxygen pressures of 75% and below (for which range we have data from both the control series and the series with ethylene), the four highest rates of CO_2 production (for the last three intervals) and the combinations that gave them are: 5.08 mg. (30° and 75% without ethylene); 4.92 mg. (30° and 75% with ethylene); 4.44 mg. (25° and 50% without ethylene); 4.22 mg. (30° and 75% with ethylene).

DERIVED GRAPHS OF ETHYLENE EFFECT
ON CO₂ PRODUCTION

The graphs of figure 1 show that the presence of ethylene in the gas stream accelerated CO₂ production in some tests, retarded it in others, and exerted no considerable influence on this process in still other tests. It is also obvious that the nature of the ethylene effect was related to oxygen percentage in a definite way, while its absolute amount was related to oxygen percentage, temperature, and time. (The main oxygen-pressure relations of this ethylene effect were briefly presented by LIVINGSTON and MACK before the American Society of Plant Physiologists at New Orleans, December, 1931). A convenient way to bring out the relations of apparent ethylene effect to time, temperature, and oxygen pressure is to employ the plus and minus percentages shown in the last columns of tables II-V. These are our indices of ethylene effect, which will next be considered with respect to both sign and magnitude. Sufficient reduction of a positive value of course leads to the zero value, and still further reduction gives progressively *increasing* negative values; considered in this way, acceleration and retardation are brought into a single continuous series of magnitudes.

Comparison of corresponding values of the index of ethylene effect upon CO₂ production in successive observation intervals shows no consistent variation with respect to time (that is, to the advancing development of the seedlings). We shall consequently refer here, as before, only to the data for the last three intervals taken together. These are shown in table VII and by the continuous-line graphs of figure 2. The plus and minus percentages are ordinates in both parts of that figure. The left-hand part consists of five graphs, one for each tested temperature, and abscissas are oxygen-pressure values. The right-hand part consists of eight graphs, one for each tested oxygen percentage, and abscissas are temperature values. Each graph is accompanied by a horizontal line to represent the zero value of this index, positive values (acceleration of CO₂ production) being of course plotted above the line while negative values (retardation) are plotted below it. Only the scale of abscissas is shown, at the bottom of each part of the figure, but the ordinate value for each

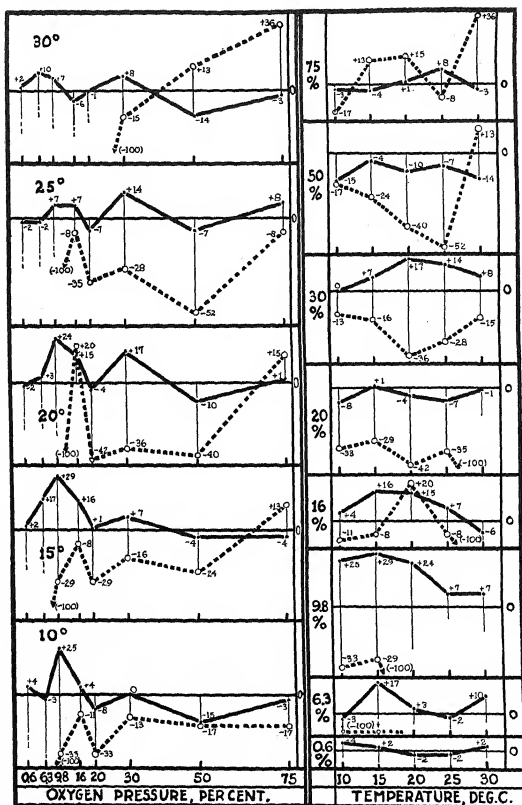


FIG. 2.—Plus and minus percentages of ethylene effect on CO₂ production (continuous lines) and shoot elongation (broken lines). Ordinate values are inscribed on the graphs. At left, five pairs of oxygen-pressure graphs; at right, seven pairs of temperature graphs. A common zero-line is shown for each pair. Data are from table VII.

point is inscribed near the point. Like ordinate scales are used for all graphs. (The broken lines of figure 2 represent percentage effects of ethylene on shoot elongation, to which reference will be made farther on.)

On the oxygen-pressure graph for 20° (left-hand part of figure 2), which may be taken as a typical example, ethylene effect is shown as negligible (-2%) for the lowest oxygen pressure (0.6%). The graph ascends and reaches a first maximum ($+24\%$) for oxygen pressure of 9.8% . It then descends to a second minimum, showing a slight retardation (-4%) for oxygen pressure of 20% . It then ascends to a second maximum, with acceleration of $+17\%$, for oxygen pressure of 30% . It descends again to a third minimum, for oxygen pressure of 50% , with retardation of -10% ; and it ends with the point for oxygen pressure of 75% , for which the ethylene effect is shown as negligible ($+1\%$).

The corresponding oxygen-pressure graphs for the remaining four temperatures are shaped essentially like the one for 20° , just described, but some quantitative differences that may be definitely related to temperature may be mentioned. The first maximal point has the abscissa 9.8 for 10° , 15° , and 20° but its abscissa may be somewhat larger for 25° and it is shown as 6.3 for 30° . The second minimal point has the abscissa 20 for 10° , 15° , 20° , and 25° but its abscissa is shown as 16 for 30° . The second maximal point is shown with the abscissa 30 for all five temperatures. The abscissa of the third minimum is shown as 50 for all temperatures excepting 15° , for which evidence is inconclusive. Whether the final point, with abscissa 75, is to be regarded as a third maximum is of course not clear, since available data do not extend beyond the oxygen pressure of 75% , but that point is considerably higher on the scale of ordinates than is the third minimum, in all instances excepting the graph for 15° , on which these two ordinates are alike (-4%).

Considering that the numerical results are surely not free from deviations, and considering the remarkable similarity among all the oxygen-pressure graphs of ethylene effect on CO_2 production (of which the continuous lines at the left of figure 2 are a summary), there seems to be no doubt that this remarkably consistent and regular relation between ethylene effect and oxygen pressure must be

regarded as a very real thing, at least within the parameter limits of this study. The effect produced by the introduction of ethylene into the environment of our cultures was clearly dependent on the current pressure of oxygen in the gas stream. For all temperatures tested ethylene treatment was generally *without considerable effect* with oxygen pressure about 0.6%; it generally gave pronounced *acceleration* with oxygen pressure about 9.8%; it generally produced some *retardation* (or no considerable acceleration) with oxygen pressure of about 20%; it generally gave considerable *acceleration* with pressure of about 30% and a similar *retardation* with pressure of about 50%; with oxygen pressure of about 75% the effect was generally negligible.

So far as we are aware, no such relation as this has hitherto been described in published accounts, although this relation is obviously in part somewhat nearly parallel with MACK'S (32) double optimum relation between oxygen pressure and CO₂ production by these seedlings in the absence of ethylene, which relation is now broadened to apply also to the tests with ethylene. The first maximum and the second minimum on a primary oxygen-pressure graph (fig. 1) have about the same abscissas, respectively, as are shown for the first maximum and the second minimum on the corresponding secondary oxygen-pressure graph of ethylene effect on CO₂ production (continuous lines, figure 2, left-hand part). This correspondence is not precise, but we may say, in general, that oxygen pressure of about 9.8%, which was specially suited to CO₂ production in both series, was also specially suited to the development of ethylene acceleration of that process; while pressure of about 20%, which generally allowed only low rates of CO₂ production in both series, also allowed only negligible ethylene effect.

No generalization that would apply to all oxygen pressures seems to be possible with regard to the manner in which ethylene effect on CO₂ production varied with temperature. Reference to the continuous-line temperature graphs of figure 2 (right-hand part) indicates that: (a) Temperature appears to have had little or no influence on ethylene effect for the lowest and highest oxygen pressures (0.6 and 75%), also for the intermediate pressure of ordinary air (20%). (b) With pressure of 50% all tested temperatures show negative effects (ethylene retardation of CO₂ production), but these are large

only for 10° , 20° , and 30° . (c) With pressure of 9.8% the effect is shown as positive (ethylene acceleration of CO_2 production) for all tested temperatures, but it is very great for 10° , 15° , and 20° and small for 25° and 30° . (d) Pressures giving different kinds of ethylene effect according to temperature are: 6.3% (negligible for 10° , 20° , and 25° , positive and great for 15° , positive and considerable for 30°); 16% (about negligible for 10° and 30° , positive and great for 15° and 20° , positive and small for 25°); and 30% (as for 16% but with greater effect shown for 20° and 25° instead of 15° and 20°).

Considering degrees of ethylene acceleration of CO_2 production, it appears that the temperature range giving the greatest acceleration for any oxygen pressure tended to shift upward on the thermometer scale as oxygen pressure was greater. The greatest acceleration percentage (24–29%) is shown for 10° , 15° , and 20° with oxygen pressure of 9.8%, but marked acceleration (14–17%) is shown for 15° with pressure of 6.3%, for 15° and 20° with pressure of 16%, and for 20° and 25° with pressure of 30%.

Considering degrees of ethylene retardation of CO_2 production, no very pronounced negative effects are shown excepting for oxygen pressure of 50%, with which the greatest retardations amounted to from 10 to 15%. But it seems to be very significant that the oxygen pressure of ordinary air (20%) showed no considerable ethylene acceleration of this process at any temperature, and did show some small but probably considerable retardations at 10° , 20° , and 25° .

Many rather inadequately planned tests of ethylene effect on plant processes have been performed from time to time, by numerous experimenters, usually with ethylene mixed with ordinary air, and it is therefore interesting to note that our results show no very marked acceleration of CO_2 production with such a gas mixture. Had our tests, with 0.1% of ethylene in the gas stream, been confined to an oxygen pressure of 20% and to the ordinary temperatures of 20° – 25° , our conclusion would probably have been that ethylene produced no acceleration of CO_2 production and seemed to retard it more or less, as much as 7% at 25° . Such a conclusion would of course be true for the special conditions referred to, but would be very far from representing the general relations of ethylene to CO_2 production in these seedlings.

Experiments performed with inclosed and stagnant bodies of gas whose constitution with regard to oxygen and ethylene is known only for the beginning of the exposure period may perhaps be of some value in the empirical development of ethylene treatments for horticultural or commercial purposes (that is, to hasten or modify the ripening, etc., of plant products or to promote the sale of ethylene), but such naïve and "practical" experiments can surely lead to nothing but confusion when scientific interpretation is attempted on the

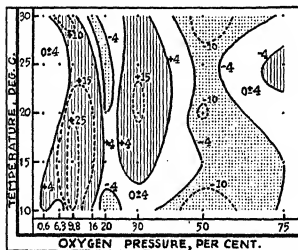


FIG. 3.—Three-dimensional diagram showing plus and minus percentages of ethylene effect on CO_2 production. Unshaded areas represent negligible effect (percentages between -4 and $+4$); stippled areas represent retardation (percentages below -4); hachured areas represent acceleration (percentages above $+4$). Data are from table VII.

present report is of course no more than a very simple and small beginning; further studies, with suitable plant material, adequate controls, and sufficiently precise experimental and logical technique will doubtless broaden our field of vision in this direction and may be expected to reveal some of the shortcomings of the present study.

The plus and minus percentages of ethylene effect on CO_2 production in the last three intervals are set forth in another way by the three-dimensional diagram of figure 3, which shows the relations of ethylene effect to both oxygen pressure and temperature. In the plane of the paper, vertical coordinates are temperatures and hori-

basis of rule-of-thumb procedures derived from such experimentation. Commercial and practical methods for the ethylene treatment of plant material certainly need to be developed and improved *ad hoc*, but the outcome of the limited study here reported indicates that we must await the development of fundamental science before useful rationalization concerning the complex causal influences involved here may be applicable at all. The

zontal coordinates are oxygen pressures, while the percentage indices of ethylene effect are represented by coordinates supposed to rise perpendicularly from the plane of the paper. The locations of the points of observation are shown by dots and the included plane surface is subdivided by contours (after the fashion of a topographic chart) into three kinds of areas. The regions without shading represent approximately negligible values of ethylene effect (from -4 to $+4$), the regions marked by stippling represent retardation (index values below -4), and those marked by hachures represent acceleration (index values above $+4$). Comparison of this diagram with the two-dimensional graphs of figure 2 and with the statements derived from them will help to show the complex relations that are indicated; of course these two figures show the same relations, for they are merely different plottings of the same set of numerical data.

PRIMARY GRAPHS OF SHOOT ELONGATION

The average values for shoot elongation, that is, the mean length of the longest ten shoots at the end of each test, are shown graphically in figure 4, the numerical data being taken from table VI. Continuous lines represent the tests without ethylene and broken lines represent those with ethylene. Ordinates are the growth averages in question and their numerical values are inscribed near the points of observation as plotted. The left-hand part of the figure consists of five pairs of graphs, a pair for each tested temperature, and abscissas are oxygen pressures. At the right of each control graph a short segment is added to show the approximate slope beyond the point for oxygen pressure of 75%, these indications being derived from MACK's earlier paper. The right-hand part of figure 4 consists of seven pairs of graphs, a pair for each tested oxygen pressure, and abscissas are temperatures. Although the growth data are derived from measurements much less precise than were the measurements of CO_2 production, yet the consistency of the several graphs of this figure indicates a high degree of reliability.

The continuous-line graphs, for the control series, without ethylene, show definite relations between shoot elongation, oxygen pressure, and temperature, as MACK has already pointed out. With the exception of the one for 30° , each of the oxygen-pressure graphs for

the control series (continuous lines, left-hand part of figure 4) shows a maximal point or region with abscissa about 50%. This is MACK's lower or secondary pressure optimum for growth. A first minimal

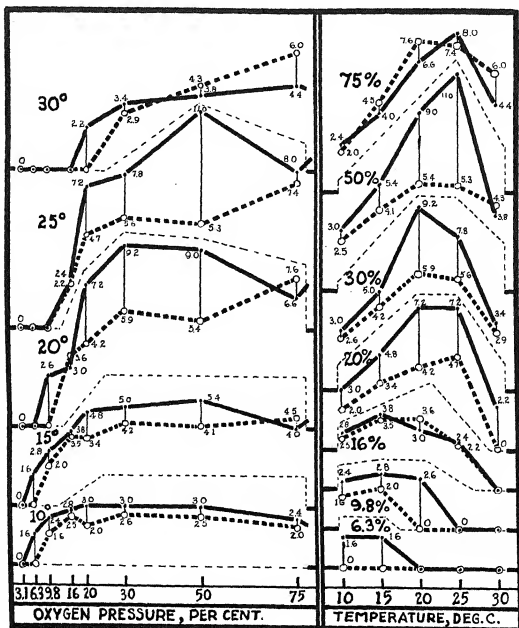


FIG. 4.—Graphs of shoot elongation without ethylene (continuous lines) and with ethylene (broken lines). At left, five pairs of oxygen-pressure graphs; at right, seven pairs of temperature graphs. A common zero-line is shown for each pair and the index values are inscribed on the graphs. Data are from table VII.

point (with index value of zero) appears at the left end in every case and this point shifts to the right with higher temperature.

Excepting on the graph for 30° , the ordinate at the extreme right (for pressure of 75%) is smaller than that for pressure of 50%, and MACK's data show that this point for pressure of 75% is a second graph minimum on the graphs for 15° , 20° , and 25° . The upper or main oxygen-pressure optimum for shoot elongation is 95%.

Each of the broken-line graphs shown in the left-hand part of figure 4, for the series with ethylene, lies generally below the continuous line of the same pair, which shows that the presence of ethylene in the gas stream, at a maintained pressure of 0.1%, retarded shoot elongation in nearly every instance. These differ from the corresponding continuous lines in that they fail to indicate either the first graph maximum or the second graph minimum. The latter is perhaps suggested by the graph for 10° , but the slope is generally upward throughout. The ethylene-treatment graphs for 10° and 15° each show what may perhaps be a significant sag in the region of pressure about 20%, a sag that is not shown by the corresponding control graphs nor by the other oxygen-pressure graphs for ethylene treatment. The graph pairs for 20° and 25° show exceptionally great ethylene retardation for oxygen pressures of 20, 30, and 50%, and the two graphs of each of these pairs consequently diverge widely in the region of these three pressures.

Without ethylene, the oxygen-pressure range that gave greatest growth at any tested temperature (fig. 4, left-hand part) tended to have higher pressure limits as the temperature was higher, but this cannot be said of the series with ethylene. As has been noted, MACK's secondary pressure optimum for growth failed to appear when ethylene was used, and the greatest pressure tested with ethylene (75%) gave, at each tested temperature excepting 10° , the greatest growth index for the given temperature. At 10° the optimal pressure range is shown as 30–50% without ethylene, but both graphs for this temperature may perhaps be regarded as nearly horizontal throughout the whole range from 16 to 75%. What might have been shown by ethylene treatment with oxygen pressures above 75% is of course not brought out.

The lowest tested oxygen pressure that gave measurable growth with ethylene at any tested temperature also tended to be greater as the temperature was higher, and this critical pressure with ethyl-

ene was generally one interval higher on our pressure scale than was the corresponding lowest pressure showing measurable growth without ethylene. The graphs for 25° are an exception to this statement, however, for 16% was the lowest tested pressure permitting measurable growth at that temperature, with ethylene as well as without ethylene. With that exception, the minimal oxygen requirement for shoot elongation was evidently greater with ethylene than without it and greater at higher than at lower temperatures.

No maximal oxygen pressure for shoot elongation at any tested temperature is shown for either the control series or the one with ethylene. If such a maximal pressure might be found it would probably be above the pressure of 100%, for both series and for all five temperatures,—surely above 75% for the series with ethylene and above 98.3% for the series without ethylene. If we suppose a maximal pressure for growth as above 100% (pure oxygen), the same logical difficulties are introduced as arose from the query about maximal oxygen pressures for CO₂ production.

Turning to the temperature graphs (right-hand part of figure 4), the temperature optimum for shoot elongation tended, in both series, to be higher as the oxygen pressure was greater. For pressure of 9.8% it is shown as 15° for both series. For pressure of 16% it is shown as 15° for the control series and as 15°–20° for the series with ethylene. For greater pressures it is shown for both series as 20° or 25°, or between these temperatures.

The lowest temperature to give measurable growth with ethylene was evidently below 10° for all tested oxygen pressures from 9.8 to 75% inclusive. Pressures of 0.6 and 6.3% failed to permit measurable growth with ethylene at any tested temperature, and it will be recalled from MACK's report that oxygen pressures of 0.6 and 3.1% without ethylene failed in the same way.

The highest tested temperature permitting measurable growth was evidently above 30° for all tested oxygen pressures greater than 16% without ethylene, and for all tested pressures greater than 20% with ethylene. For smaller pressures this highest temperature was lower as the pressure was smaller. It was also generally lower with ethylene than without it. For 20% it was 25° with ethylene but above 30° without ethylene; for 16% it was 25° for both treatments;

for 9.8% it was 15° with ethylene and 20° without ethylene; with 6.3%, 10° and 15° gave some growth without ethylene but neither of these temperatures nor any other tested temperature gave any measurable growth with ethylene; for 0.6% no tested temperature permitted measurable shoot elongation either with ethylene or without it.

With regard to the absolute values of the indices of shoot elongation, the highest index here considered is 11.0 (for 25° and 50%) in the control series, but the highest value in the series with ethylene treatment is only 7.6 (for 20° and 75%). Of course the lowest index is zero in both series, and many combinations of temperature and

WITHOUT ETHYLENE			WITH ETHYLENE		
SERIAL NO.	GROWTH INDEX	COMBINATION	SERIAL NO.	GROWTH INDEX	COMBINATION
1	11.0	25°, 50%	1	7.6	20°, 75%
2	9.2	20°, 30%	2	7.4	25°, 75%
3	9.0	20°, 50%	3	6.0	30°, 75%
4	8.0	25°, 75%	4	5.9	20°, 30%
5	7.2	20°, 20%	5	5.6	25°, 30%
		25°, 20%	6	5.4	20°, 50%
			7	5.3	25°, 50%
			8	4.7	25°, 20%
			9	4.5	15°, 75%
			10	4.3	30°, 50%
			11	4.2	20°, 20%
					15°, 30%

oxygen pressure that gave no measurable shoot elongation with ethylene gave considerable elongation in the absence of that compound. If for each series we consider as "good" for shoot elongation the combinations whose indices fall in the upper third of the total index range for the series, we find that there are four such good combinations for the control series while there are seven for the series with ethylene treatment. The two "good" index ranges do not overlap, however; 8.0 is the smallest growth index in the "good" group without ethylene and 7.6 is the greatest index in the other "good" group. These "good" combinations are shown above, arranged in the descending order of their indices (whose values are also

shown), and each series is continued to include the combinations 20° with 20% and 25° with 20%, which are specially interesting because they may be most frequently encountered in nature at times when plants are active. These are shown in bold-face type. Indices below the horizontal line fall in the "medium" class of their own series; that is, in the middle third of the index range. The four "good" combinations without ethylene occur also among the "good" ones with ethylene, but with marked shifting of their order, and the three additional "good" combinations in the series with ethylene all fall in the "medium" class in the control series. The highest index for oxygen pressure of 20% (that of ordinary air) without ethylene is 7.2 (20° and 25°), and the corresponding highest indices for the same pressure with ethylene are 4.7 (25°) and 4.2 (20°). These combinations fall in the "medium" class in both series.

It is surely significant that, with respect to shoot elongation, higher oxygen pressures so greatly surpassed the pressure of ordinary air, both with and without ethylene. With respect to the rapidity of shoot elongation in the first few days after the beginning of germination, our lot of wheat seed was apparently well suited to temperatures frequently occurring naturally but it was apparently not well suited to any oxygen pressure ever encountered in nature; natural atmospheric oxygen pressure might be considered as at least 60% deficient for these seedlings, so far as shoot elongation is concerned. Of course such a statement is based on and limited by the background conditions of these experiments.

Although the available data on shoot elongation with oxygen pressures above 75% (90, 95, and 98.3%) cannot be considered in our discussions of ethylene effect, it may be added here (32, table V) that the series without ethylene furnished just one index value higher than 11.0 (namely, 12.2 for 25° and 95%), and also five additional values below 11.0 but still in the "good" class.

DERIVED GRAPHS OF ETHYLENE EFFECT ON SHOOT ELONGATION
AND THEIR COMPARISON WITH CORRESPONDING GRAPHS
OF CO₂ PRODUCTION

The plus and minus percentages that represent effects of ethylene on shoot elongation (tables VI and VII) are shown by the broken-line graphs of figure 2, which are so arranged that each one may

readily be compared with the corresponding continuous-line graph of ethylene effect on CO_2 production. The same zero line is used for both graphs of each pair. Ordinates are the percentages, whose numerical values are inscribed on the graphs, and abscissas are shown at the bottom.

As has been remarked, the ethylene treatment employed generally retarded shoot elongation, but acceleration of that process occurred in five instances; namely, for oxygen pressure of 16% combined with 20° (+20), for 50% combined with 30° (+13); and for 75% combined with 15° (+13), with 20° (+15), and with 30° (+36). All of the remaining tested combinations of temperature and pressure showed considerable retardation due to ethylene, the percentage index nearest to zero being -8, which appears for three different combinations. Aside from the ultimate value of -100, the numerically largest negative index is -52. The total range of these percentages is from -100 to +36.

The oxygen-pressure graphs (broken lines, left-hand part of figure 2) of ethylene effect on growth at 10°, 15°, 20°, and 25° are remarkably similar in form and many of their features are in close agreement with the corresponding graphs of ethylene effect on CO_2 production. To appreciate this it is desirable to neglect the zero line and consider a single scale of ordinates, as though -29 and +29, for example, were given as 0.71 and 1.29 respectively, acceleration being thus denoted by values greater than unity and retardation by values smaller than unity; the actual drawing would be the same as is shown in the figure, however. Each of these four graphs of ethylene effect on growth shows a first maximum for pressure of 16% and a second minimum for pressure of 20% (the *first* minimum being at the extreme left). A second maximum, for 30%, is at least generally indicated, as is also a third minimum, for 50%. A third maximum, for 75% or above, is clearly shown for all tested temperatures excepting 10°.

For the lower three temperatures the most striking difference between these graphs and the corresponding oxygen-pressure graphs for ethylene effect on CO_2 production (continuous lines, left-hand part of figure 2) is that the first graph maximum for effect on growth corresponds to a pressure of 16%, while the first graph maximum

for effect on CO_2 production corresponds to a pressure of 9.8 %. For the graph pair for 25° a similar difference is indicated.

Each of these pressure graphs of ethylene effect on shoot elongation shows a definite and pronounced minimum for pressure of 20%. There was apparently something about this oxygen pressure of ordinary air that generally made for characteristically low rates of CO_2 production (both with and without ethylene) and for characteristically low ethylene effect on both CO_2 production and shoot elongation. It is suggested, as has been noted, that this same pressure also made for a somewhat retarded rate of shoot elongation at 10° and 15° with ethylene, but this suggestion does not appear for shoot elongation without ethylene nor at the three higher temperatures with ethylene. When we say "low ethylene effect" here it is of course understood that any negative value of the index of this effect is considered as lower than any positive value, etc., and that a lowering of effect in this sense may *decrease acceleration*, may *change acceleration to retardation*, or may *increase retardation*.

Turning to the temperature relations of ethylene effect on shoot elongation, no clear generalizations are to be derived from a study of the temperature graphs (broken lines, right-hand part of figure 2); nor is there any apparent general correlation between these graphs and the corresponding ones of ethylene effect on CO_2 production. It should be noted, however, that ethylene retardation of growth was great at all temperatures with pressure of 20%, and that the two temperature graphs for that pressure (which is critical for several features of this study, as just mentioned) are both rather nearly horizontal and almost alike in their departures from the horizontal excepting at the extreme right (30°). Temperature apparently exerted no clearly consistent influence upon the effect produced by ethylene, either on CO_2 production or on shoot elongation. For the pressure 16%, 20° appears to have been optimal for ethylene effect on growth, and the same temperature lies in the optimal region for the corresponding ethylene effect on CO_2 production. For pressure of 30% the two temperature graphs are of similar form but reversed; the continuous-line graph for ethylene effect on CO_2 production ascends from 10° to 20° and then descends with higher temperatures (all ordinates being positive, excepting the one for 10° , which is

zero), but the broken-line graph descends to a minimum for 20° and then ascends (all ordinates being negative). For pressure of 50% ethylene retardation of growth increased with temperature up to 25° , but this pressure at 30° gave one of the few instances of growth acceleration and the continuous-line graph (for effect on CO_2 production) shows no consistent relation to the broken-line graph.

All of our data concerning ethylene effect on shoot elongation are plotted together on the three-dimensional diagram of figure 5, which may be compared with the corresponding diagram for ethylene effect

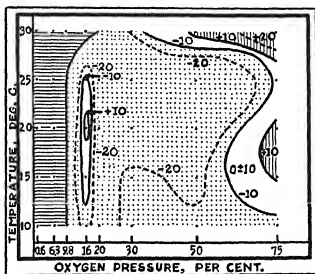


FIG. 5.—Three-dimensional diagram showing plus and minus percentages of ethylene effect on shoot elongation. Unshaded areas represent negligible effect (percentages between -10 and $+10$); stippled area represents retardation (percentages below -10); vertically hachured areas represent acceleration (percentages above $+10$). The horizontally hachured area represents combinations of temperature and oxygen pressure that permitted no growth either without ethylene or with it. Data are from table VI.

on CO_2 production (fig. 3) and with the two-dimensional graphs of figure 2. Unshaded areas represent percentage values between $+10$ and -10 ; that is, combinations of oxygen pressure and temperature that permitted little or no ethylene effect, either of acceleration or retardation. Vertically hachured areas represent ethylene acceleration of more than 10% and the stippled area represents ethylene retardation of more than 10%. The horizontally hachured area represents combinations that permitted no growth either without ethylene or with it.

It is of interest to note that the only tested combination of tem-

perature and oxygen pressure for which ethylene gave considerable acceleration of both CO_2 production and shoot elongation is the combination of 20° with oxygen pressure of 16%. With this combination the ethylene acceleration was 15% for CO_2 production and 20% for growth. But that combination gave only low indices of CO_2 production and shoot elongation, either without ethylene or with it.

It is apparent that current oxygen pressure was generally predominant in the control of ethylene effect upon both of the processes studied, as well as upon the rates of these processes themselves, and that maintained temperature was also influential, although not so regularly so. It is also apparent that the influence of ethylene on growth was intimately but not proportionately related to its influence on CO_2 production.

General conclusion

The experimental data presented in the preceding pages lead to the general conclusion that ethylene effects on our seedlings cannot be characterized in any simple manner; no definite statement about them can be made without quantitative reference to the concomitant non-ethylene conditions of oxygen pressure and temperature. It may easily be supposed that ethylene effects on living plants in general must be influenced not only by ethylene pressure, oxygen relations, and temperature relations, but also by water relations, nutrition relations, light relations, and the nature and physiological state of the tissues dealt with. Doubtless many chemical relations other than those mentioned may be influential on ethylene effects.

These remarks illustrate the more general and fundamental scientific principle of multiple causation, which is now belatedly becoming appreciated by many experimenters in physiology and other kinds of biological science. In experimental studies on the physiology of an organism it is obviously essential that real account be taken of all the influential conditions that are involved in the determination of process rates, through the definite establishment and specification of the experimental background complex as well as of the experimental variables that are considered.

Also, our results furnish an example of the manner in which what may be called second and third derivatives are now inevitably de-

manding attention in physiological studies of this sort. We shall need more and more to undertake studies on the manner in which effects, considered as due to a specified influence, may themselves be controlled through the action of still other concomitant influences. For instance, examples of possible accelerating or retarding influence of ethylene upon a plant process, such as CO_2 production or shoot elongation, are not very enlightening without reference to possible accelerating or retarding influence of current oxygen pressure upon the ethylene effects considered.

Summary

1. In this paper a review of literature on the influence of ethylene on plant processes is followed by an account of some preliminary experiments on ethylene effects. The rest of the paper reports a somewhat elaborate experimental study of the influence of temperature and oxygen pressure on the effects of a single maintained concentration of ethylene in controlling the rates of CO_2 production and shoot elongation in very young wheat seedlings. This study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University.

2. From the literature and from the results of the preliminary experiments, as well as from considerations of the nature and control of plant processes in general, it seemed that the many apparent discrepancies in observed ethylene effects on plants might be related to influences of non-ethylene conditions that must be effective along with ethylene influence. With this seemingly obvious, although generally unexpressed, thought in mind, an attempt was made to plan and carry out a relatively complete series of consistently comparable experimental tests. There were four experimental variables: maintained temperature, maintained oxygen pressure, time, and the presence or absence of ethylene at a single concentration or pressure. The background conditions of the environment, essentially alike for all tests, were established by using a standard 3-salt nutrient solution as culture medium (in which the seedlings were submerged), by the continuous bubbling of a synthetic gas mixture through the medium, and by the absence of light in the culture chambers. Nutrient solutions were not renewed. The gas stream maintained the

partial pressures of oxygen and nitrogen, or of these two gases and ethylene, and continuously removed CO_2 as well as any other volatile products of metabolism that may have been eliminated by the seedlings. There were 12 different gas mixtures without ethylene (containing 0.6–98.3% of oxygen by volume) and 8 different mixtures with ethylene (containing 0.6–75% of oxygen by volume). When ethylene was present its pressure was 0.1% by volume. Five different maintained temperatures were employed, 10° , 15° , 20° , 25° , and 30° . Five consecutive observation intervals in the experimental period gave five increments of CO_2 production in each experiment. Each experiment was repeated and some were several times repeated.

3. A relatively high degree of uniformity in the wheat seedlings used was secured by the employment of a standard germination procedure, with a 42-hour germination period, a maintained temperature of 19.5° , and a continuously flowing stream of air through water in which the seedlings were submerged; also by selecting the seedlings used out of a much larger number. One hundred seedlings were used in each test.

4. Carbon-dioxide increments were absorbed and measured by titration at the ends of the observation intervals and shoot elongation was measured as a single increment at the end of the entire experimental period. The experimental period was 48 hours, but the first 2 hours of it was allowed for seedling transfer and adjustment, and CO_2 measurements extended throughout the last 46 hours only.

5. The numerical results dealt with are: (a) average hourly rates of CO_2 production, for 300 different combinations of temperature, oxygen pressure, and time without ethylene, and 200 similar combinations with ethylene; (b) indices of shoot elongation for 60 different temperature-oxygen combinations without ethylene and 40 different combinations with ethylene, all these growth indices referring to the entire experimental period. These data are presented in tables and by means of graphs. Ethylene effects on CO_2 production and shoot elongation were computed as plus or minus percentages, on the basis of the corresponding controls without ethylene, and the resulting indices of ethylene effect are also presented in tables and graphs. Attention is given to the relations between ethylene effect on CO_2 production and ethylene effect on shoot elongation; as well as to the

influence of oxygen pressure, temperature, and time on ethylene effect.

6. The experiment series without ethylene was completed before the series with ethylene was begun and its main results have been previously reported by MACK (32). The new data presented in the present paper refer to the ethylene series, MACK's data being employed as controls for the study of ethylene effects.

7. The more obvious relations apparently brought to light by this study may be summarized as follows:

Time not an effective variable with respect to CO₂ production within the limits of this study.—Relations between oxygen pressure and temperature on the one hand, and CO₂ production on the other, and the influence of ethylene on these relations, are most clearly shown by the data for the later observation intervals (when the seedlings were slightly more advanced in development), but there are no marked and consistent differences that can be related to time, aside from such differences as are connected with the larger CO₂ increments secured for the later intervals. Consequently the mean rates of CO₂ production for the last three observation intervals combined, which represent the last 36 hours of the experimental period, are mainly employed in our discussions.

Double optimum of oxygen pressure for CO₂ production.—MACK's double optimum of oxygen pressure for CO₂ production is generally indicated for the experiment series with ethylene as well as for the series without it. This constitutes a pronounced confirmation of the reality of the double optimum, for the two series were independent but essentially alike with respect to oxygen pressures up to 75%, and they were planned to differ only with respect to ethylene.

Lower optimum of oxygen pressure for CO₂ production and the abscissal position of the corresponding secondary graph minimum.—A typical oxygen-pressure graph of CO₂ production ascends to a first maximal point (whose abscissa is MACK's lower optimum of oxygen pressure), then descends to a second minimal point, and then ascends again toward the second maximal point (whose abscissa is the main pressure optimum). The abscissal positions of the first two of these critical points vary somewhat. It is suggested that these points tend to shift to the right (toward higher oxygen pressure) as the tem-

perature is higher and also as an apparent ethylene effect. The abscissa of the first graph maximum varies between 6.3 and 16% and the abscissa of the second graph minimum varies between 9.8 and 20%. The second minimum lies to the right of the first maximum by from 4 to 10 percentage units of oxygen pressure.

Main oxygen-pressure optimum for CO₂ production.—At all tested temperatures the main optimal oxygen pressure for CO₂ production was about 90–95% for the control series (as previously shown by MACK), and it is indicated as surely above 75% for the series with ethylene.

Minimal and maximal oxygen pressures and temperatures for CO₂ production.—No minimal or maximal oxygen pressure for CO₂ production appeared at any tested temperature, either without ethylene or with it; neither was any minimal or maximal temperature for CO₂ production shown for any tested oxygen pressure.

Optimal combinations of temperature and oxygen pressure for CO₂ production.—Without ethylene, the temperature-pressure combination that gave the highest rate of CO₂ production in the last three intervals together was 30° and 95%. That combination gave a mean hourly rate of 5.86 mg. for 100 seedlings. The only other combinations giving corresponding rates above 5.00 mg. were 30° combined with 90% (5.75 mg.) and 30° combined with 98.3% (5.31 mg.). For the series with ethylene, the highest mean hourly rate of CO₂ production in the last three intervals was given by the combination 30° and 75% (4.92 mg.). But the series with ethylene included no oxygen pressures above 75%, and if pressures higher than 75% are not considered in the control series, then the best combination for that series is also 30° and 75% (5.08 mg.).

Optimal oxygen pressures for shoot elongation.—For shoot elongation without ethylene there were generally two oxygen-pressure optima (as MACK has pointed out), a lower one about 50% and a higher one about 95%. Because the series with ethylene did not extend beyond the pressure of 75%, our data do not actually show the second of these pressure maxima for that series, although the probable occurrence of that maximum is indicated by the fact that pressure of 75% generally gave for any temperature a growth value higher than that given by any smaller pressure. The first or lower pressure op-

tinum (about 50%) is not shown at any temperature for the series with ethylene.

Minimal oxygen pressures for shoot elongation.—The minimal oxygen pressure for shoot elongation at any temperature, which varied (for both series) from below 6.3% (10°, without ethylene) to about 20% (30°, with ethylene), was evidently greater at higher temperature than at lower, and also greater with ethylene than without it.

Maximal oxygen pressures for shoot elongation.—No maximal oxygen pressure for shoot elongation at any temperature is shown for either the control series (for which it was evidently above 98.3%) or for the series with ethylene (for which it was evidently at least above 75%).

Optimal temperatures for shoot elongation.—Both without ethylene and with it, the optimal temperature range for growth with any tested oxygen pressure was about 15°–20° for pressures of 9.8 and 16%, and about 20°–25° for pressures of 20% or above.

Minimal temperatures for shoot elongation.—The minimal temperature for shoot elongation was evidently below 10° for all tested oxygen pressures above 6.3%, both without and with ethylene, and also for 6.3% without ethylene. But no measurable growth occurred at any tested temperature for pressure of 0.6% in either series, nor for pressure of 3.1% without ethylene, nor for pressure of 6.3% with ethylene.

Maximal temperatures for shoot elongation.—The maximal temperature for shoot elongation was evidently above 30° for all oxygen pressures greater than 16% without ethylene and for all pressures greater than 20% with ethylene. For smaller pressures this critical temperature was lower as the pressure was smaller and generally lower with ethylene than without it.

Optimal combinations of temperature and oxygen pressure for shoot elongation.—For shoot elongation the best of the tested combinations of temperature and oxygen pressure without ethylene was 25° with 50%; this gave a growth index of 11.0. With ethylene the best combination was 20° with 75%, which gave an index of 7.6. The four combinations that gave largest growth indices (11.0 to 8.0) in the control series occur among the seven combinations that gave largest indices (7.6 to 5.3) in the series with ethylene. Each of the two index

ranges just mentioned embraces the upper third of the total range for its series. These seven "best" combinations are all for 20° or 25° combined with oxygen pressure of 30, 50, or 75%.

* *General effect of ethylene on CO₂ production.*—The influence of ethylene on CO₂ production at any temperature varied in a remarkably complex but very consistent manner, according to the current oxygen pressure. At all tested temperatures it was *negligible* or nearly so for very small oxygen pressure (about 0.6%) and also for the greatest pressure tested with ethylene (75%). But the presence of ethylene generally *accelerated* CO₂ production for each of two intervening but widely remote ranges of oxygen pressure (about 10 and 30%), and it *retarded* this process, or else was without considerable effect, for the oxygen pressure of ordinary air (20%) and for oxygen pressure of 50%.

Optimal combinations of temperature and oxygen pressure for ethylene acceleration of CO₂ production and for ethylene retardation of the same process.—The most pronounced ethylene acceleration of CO₂ production (amounting to 29%) occurred with the combination of 15° with oxygen pressure of 9.8%, but the combinations of that same oxygen pressure with 10° and with 20° gave nearly as pronounced accelerations (amounting to 25 and 24% respectively). The most pronounced ethylene retardations of CO₂ production occurred with pressure of 50% combined with 10°, 20°, and 30° (amounting to 15, 10, and 14% respectively). With that same pressure of 50%, 15° and 25° showed smaller retardations (amounting to 4 and 7% respectively). Retardations amounting to 7 and 8% were shown by oxygen pressure of 20% combined with 10° and with 25°.

{ *Effect of ethylene on shoot elongation.*—The influence of ethylene on shoot elongation was retardation for nearly all tested combinations of temperature and oxygen pressure, and the nature and degree of this influence was clearly related to the current oxygen pressure and to the concomitant influence of ethylene on CO₂ production. At 10°, 15°, 20°, and 25°, growth retardation was relatively small, or there was acceleration, for oxygen pressures of 16, 30, and 75%, and the growth index was generally relatively great for oxygen pressures of 20 and 50%. At 30° oxygen pressures of 20% and below failed to permit measurable growth with ethylene, but pressure of 30% gave retardation and pressures of 50 and 75% gave acceleration.

Oxygen pressure of ordinary air.—It is specially noteworthy that the oxygen pressure of ordinary air showed peculiar relations. It not only induced characteristically low rates of CO_2 production, both without ethylene and with it, but also gave most pronounced ethylene retardation of shoot elongation and little or no ethylene effect on CO_2 production.

Ethylene acceleration of growth.—Ethylene acceleration of shoot elongation occurred for 15° combined with oxygen pressure of 75%, for 20° combined with 16 and with 75%, and for 30° combined with 50 and 75%.

General temperature relations of ethylene effects on CO_2 production and on shoot elongation.—Although the temperature relations of ethylene effect on CO_2 production and on shoot elongation appear to be characteristically different for different tested oxygen pressures, yet no clear generalizations seem to be possible concerning these temperature relations; to appreciate this the graphs need to be consulted. Within the limits of our tests, oxygen pressure of 20% showed little or no influence of temperature upon ethylene effect, either upon CO_2 production or upon shoot elongation.

Ethylene acceleration of both CO_2 production and growth.—The only tested combination of temperature and oxygen pressure that gave considerable ethylene acceleration of both CO_2 production and shoot elongation is the combination of 20° with 16%. This acceleration amounted to 15% for CO_2 production and 20% for shoot elongation.

Generalization from main results.—For our seedlings and for the background conditions of our experiments, current oxygen pressure was generally predominant in the control of ethylene effect upon both CO_2 production and shoot elongation, as well as upon the rates of these processes themselves, either without ethylene or with it; current maintained temperature was also influential, although not so regularly so; and the influence of ethylene on shoot elongation was intimately but not proportionately related to its influence on CO_2 production.

General conclusion.—The results of this study furnish an illustration of the principle of multiple causation, according to which many influential conditions or effective factors may take part in the determination of physiological effects, which are often popularly attributed to some single influence. No scientifically useful conception

of ethylene influence on plants can be formed without the specification of the non-ethylene conditions that operate along with ethylene in each experimental test. In other words, these results illustrate how the influence of one condition may be modified through the concomitant action of other conditions.

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STRUCTURAL AND METABOLIC AFTER-EFFECTS OF SOAKING SEEDS OF PHASEOLUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 441

WILLIAM MARSHALL BAILEY

(WITH THREE FIGURES)

Introduction

The purpose of this investigation was to determine the influence which certain physiological conditions of the seed may exert upon subsequent development of the plant. The work was concerned mainly with the effects produced by soaking the seeds for a prolonged period in distilled water previous to planting them. KIDD and WEST first gave prominence to this problem by their experimental studies (4), and by an extensive review of the literature on related investigations (5-8). In this review were considered the effects of the conditions under which the parent plant had grown, the degree of ripeness, the conditions operating during germination and early seedling stages, and the effects of soaking the seeds in water and certain salt solutions in determining the subsequent course of development. The evidence in general seems to indicate that the factors which influence a plant during its earliest stages of development have a more or less pronounced effect upon its subsequent life history.

In their experiments, KIDD and WEST (4) soaked seeds under 4 cm. of distilled water at an average temperature of 17° C. for periods varying from 8 to 72 hours. Their report does not indicate that any provision was made for the elimination of bacteria or for aeration. The seeds used were *Phaseolus vulgaris* (dwarf bean), *Pisum sativum* (culinary pea), *Vicia faba* (broad bean), *Lupinus albus* (white lupine), *Helianthus annuus* (sunflower), *Triticum vulgare* (wheat), *Hordeum sativum* (barley), *Avena sativa* (oats), and *Brassica alba* (white mustard). The seeds were germinated on damp sand in porous flower pots covered with glass. The results showed that soaking the seeds more than 24 hours diminished the rate of germination in *Pha-*

seolus, *Pisum*, and *Helianthus*, and that there was a decrease in the rate of germination in *Hordeum* resulting from 72 hours of soaking.

They obtained data showing the effects of soaking seeds (dwarf bean, culinary pea, barley) on subsequent growth by growing them for 12 days on damp sand. They found that the soaking had a marked effect in retarding subsequent growth of the plants. They also carried on experimental work with seeds treated with distilled water and grown for longer periods in the greenhouse. Wheat, oats, white mustard, dwarf beans, broad beans, and white lupine were used. Samples of these seeds were soaked 6, 24, 48, and 72 hours respectively, and planted in potting soil in the greenhouse. In the case of dwarf bean harvested $4\frac{1}{2}$ weeks after sowing, a period of soaking of only 6 hours had a marked predetermining influence in retarding growth, which was greatly augmented by longer periods of treatment. Soaking for 3 days killed all of the seeds. Broad bean 3 weeks after planting showed diametrically opposite results, since there was a progressive increase in the height of the plants and in the rate of germination of the seeds resulting from the soaking, which was carried on up to 72 hours. Wheat showed a progressive increase in height and dry weight of tops in the plants from soaked seeds up to 48 hours of treatment. There was also a small increase in the rate of seed germination. However, plants from seeds soaked 72 hours were smaller and less vigorous. Oats showed results similar to those for wheat.

In addition, KIDD and WEST investigated the effects of temperature on the predetermining influence of soaking seeds of dwarf bean in distilled water (9). It was found that the temperature during the seed treatment had no marked effect upon the yield, but that the deleterious influence of soaking was somewhat less at temperatures near 20° C. than at temperatures higher or lower than this.

Three main conclusions were drawn from the results of this experimental work: first, that the soaking in distilled water previous to sowing may have a marked influence upon subsequent growth of the plants; second, that a germination test cannot be relied upon as a criterion of what this influence will be; third, that the nature of this influence is strongly specific. Thus very different results may be obtained from closely related plants. While soaking the seeds in water

greatly retarded the growth of the plants in the case of dwarf bean, this treatment increased both the rate of germination and the size of the plants produced in the case of broad bean.

TILFORD, ABLE, and HIBBARD (19), employing many kinds of seeds, found that deleterious effects are produced by prolonged water-soaking. Different results were secured from closely allied plants. A short period of soaking (8 hours) was found to interfere with germination of dwarf bean seeds and had a deleterious influence on subsequent development of the plants. The data submitted give results only on germination. In three tests sterilized seeds treated 72 hours in sterilized distilled water with aeration gave germination tests of 72, 100, and 88 per cent respectively. Non-sterilized seeds treated 72 hours in distilled water with aeration gave germination tests of 14, 40, and 27 per cent in three respective tests. Non-sterilized seeds soaked in distilled water 72 hours without aeration gave 20, 0, and 0 per cent of germination in three tests. It was concluded that the injurious influence on seed germination resulting from the soaking in distilled water is due to bacterial activity, and to a deficiency of oxygen and an excessive accumulation of carbon dioxide.

RHINE (15) found that soaking wheat for a period of 22 days in sterilized distilled water reduced the rate of germination to 2 per cent, while soaking for a period of 29 days resulted in a complete failure of germination.

BARTON (1), while experimenting on autolysis in seeds, investigated the influence of soaking for long periods of time on seed germination. Seeds of navy beans, peas, corn, and wheat were used. Four-ounce bottles, fitted with two-hole stoppers and right-angled glass tubes, were connected in series. The bottles and connections were sterilized. Each series of bottles was filled with boiling water. When at room temperature, the water was saturated with carbon dioxide. Five seeds, after being sterilized and washed with sterilized distilled water, were placed on glass wool in the first bottle of the series. After periods of soaking varying in length up to 3 months, the water was forced out with sterilized oxygen. In 3 or 4 days, 60 to 100 per cent of the seeds germinated. BARTON concludes: "(1) that the capacity for germination is not destroyed by long-continued soaking in sterile media; (2) that destructive changes are not brought

about by enzymes or ferments within the seeds; and (3) that any change that may take place within the seed does not in any way affect the viability and is not accompanied by any visible manifestation."

In the present investigation, the study of the predetermining influence of soaking seeds in distilled water has been carried further, in an effort to determine whether this influence is to be ascribed entirely to bacterial action and interference with respiration, or whether other causes are operating; and to discover some of the subsequent structural and metabolic effects of this seed treatment.

Experimentation

Early Valentine beans (*Phaseolus vulgaris*) were used in the investigation, seeds of relatively high germinating capacity being secured. The seeds were carefully sorted, only sound ones of approximately uniform size and regular shape being used.

The seeds were soaked in sterilized distilled water aerated by means of the apparatus shown in figure 1. In order to secure uniform movement of the air, it was drawn through the apparatus by means of an aspirator. The volume of air used was approximately 24 liters per day. It seems evident that lack of oxygen or the accumulation of carbon dioxide was not a limiting factor in this investigation, in view of the fact that TANG's (18) results indicated that 6 liters of air per day was sufficient to give maximum results in the germination of wheat. The distilled water in flask *A* was sterilized in the autoclave, the flask being plugged with cotton, and cooled to room temperature. The cotton filters *F*₁ and *F*₂, carefully wrapped in heavy paper, were sterilized by prolonged heating in the autoclave. The remainder of the apparatus was sterilized by heating for 2 hours in steam at the temperature of boiling water, it being impossible to use the autoclave because of injury to the rubber connections by the high temperature. After sterilization the apparatus was connected up with as much precaution as possible to avoid contamination with bacteria and fungus spores from the air.

The seeds were presoaked 10 minutes in distilled water (2), after which they were put in the seed chamber *S*, which was then filled with a 0.25 per cent solution of "uspulun," to sterilize the seeds.

It was found in preliminary tests that this treatment in no way impaired germination. After 15 minutes the uspulun solution was drawn over into flask *E*, and replaced with water from flask *A* by means of suction from the aspirator. Approximately 1.5 liters of water was drawn through the seed chamber from flask *A* for the purpose of washing the seeds. Air was then drawn steadily through the seed chamber, after being sterilized by passing through the cotton

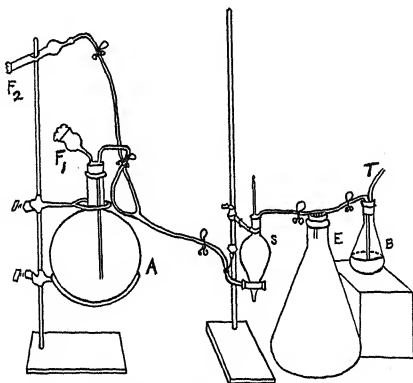


FIG. 1.—Apparatus for soaking seed with aeration: *S*, seed chamber; *A*, flask containing sterilized distilled water; *E*, large flask into which water was drawn from seed chamber; *F*₁, *F*₂, cotton filters; *B*, safety flask; *T*, tube connected to aspirator.

filter *F*₂. The water was changed four times per day by being drawn from flask *A* into the seed chamber *S* and into flask *E* by suction from the aspirator. When the water was all drawn from flask *A* it was replaced from time to time with flasks similarly filled with distilled water, sterilized and cooled, as much precaution as possible being taken to prevent contamination from the air in changing the flasks.

To test the success in the elimination of bacteria and fungi, a quantity of nutrient broth was put into a flask of the same size as

flask *E* and plugged with cotton. The flask with its contents was sterilized in the autoclave, cooled, and then connected with the apparatus in the place of flask *E*, as much precaution as possible being taken to avoid contamination from the air. A small quantity of the water in which the seeds had been soaking was drawn from the seed chamber into the broth. After incubation in a warm room for a period of 3 days, no indication of bacteria or fungi appeared in the broth. This test was made during the third day of the seed treatment.

In each experiment in which the seeds were planted, the seeds used for control were sterilized in the usual way, then washed as quickly as possible and planted at once. They are called "untreated seeds" in the report of the results.

The plants were grown in the greenhouse and were watered with the usual water supply available there. The temperature of the greenhouse usually varied between 60° and 80° F. The seeds were planted at a depth of approximately 1 inch. The plants were harvested when they had reached the blooming stage, when they were considered mature. The tops were harvested, the stems being cut at the surface of the soil.

EXPERIMENT I

The purpose of this experiment was to determine the effect of the seed treatment on germination, total number of mature plants produced, growth, average weight, time required to reach maturity, and influence of seed treatment on stem and leaf structure. The temperature at which the seeds were treated was 16°–19° C. The following seed treatments were used: no. 1, seeds untreated; no. 2, seeds treated 8 hours; no. 3, seeds treated 2 days; no. 4, seeds treated 5 days; no. 5, seeds treated 7 days; no. 6, seeds treated 9 days. This experiment was carried out between March 22 and May 9, 1930.

STEM AND LEAF STRUCTURE.—Cross-sections of the stems in the internode just above the first pair of leaves were prepared from the mature plants. The average diameter of the stem and the average thickness of the xylem and phloem in this internode were determined in the plants from the untreated seeds and in those from seeds treated 5 days. Also the ratio of the average thickness of the phloem

to that of the xylem was determined. Cross-sections of leaves from the mature plants of these two sets were also prepared. For the sake of uniformity, the leaf sections were all taken from the terminal leaflet of the uppermost mature leaf. The leaf structures of the two sets of plants were compared.

EXPERIMENT II

This experiment attempted the determination of the influence of the seed treatment on the percentage of water and dry matter in the plants produced, the abundance of various carbohydrates and nitrogen compounds, and the influence on the growth of the plants of a calcium salt in very dilute solution in the water in which the seeds were soaked. The experiment ran from November 22, 1930 to January 20, 1931. The following were the respective seed treatments: no. 1, seeds untreated; no. 2, seeds treated in sterilized distilled water with aeration 5 days; no. 3, seeds treated in sterilized distilled water containing calcium nitrate (approximately 900×10^{-6} gm. normal per liter) with aeration 5 days. The temperature was 15° – 16° C. The plants were grown in the greenhouse and harvested at maturity. The three sets of plants were harvested at the same hour of the day, 8 A.M.

Analyses for determining the percentage of dry matter, carbohydrates, and nitrogen compounds were made on samples of plants from no. 1 and no. 2. Determinations were made in duplicate. All analyses of the extractives were made within 3 weeks after harvesting. The Methods of Analysis of the A.O.A.C. (13) were generally followed in the chemical determinations.

EXTRACTION.—Not less than 20 plants (14) were ground together in a Russwin mill, and the ground material thoroughly mixed. Samples of this material were weighed off into sufficient 95 per cent alcohol, boiling hot, to make the alcohol content of the entire mass approximately 85 per cent. To this material was added 0.5 gm. of pure calcium carbonate to neutralize the acids present and thus prevent hydrolysis. The samples, in large pyrex beakers covered with watch glasses, were kept heated to the boiling temperature of the alcohol on the hot-water bath for 40 minutes. The samples were then extracted in Soxhlet extractors with 95 per cent alcohol, ether, and

water, each for 2-4 hours, then with 95 per cent alcohol over night. All alcohol used for preserving and extracting the samples was redistilled in glass, and only chemically pure ether was used. The alcohol, ether, and water used in the extraction were poured together into a large evaporating dish, and the alcohol and ether were replaced with water by evaporation. The extract was then transferred quantitatively to a volumetric flask and the volume adjusted by the addition of water. The lipid materials, together with the chlorophyll, which were precipitated out by the replacement of the alcohol and ether with water, were removed in solution in 20 cc. of chloroform. The chloroform solution was then evaporated to dryness and the weight of the substances dissolved in the chloroform determined. Since this material consists mainly of chlorophyll and the carotinoid pigments, and very little difference was found between the percentages in the plants from treated seeds and those from untreated seeds, it was assumed that there was no important difference in the quantity of these pigments in the two sets of plants.

TOTAL WEIGHT OF SOLIDS.—The total dry weight of the solids was determined by taking the sum of the dry weight of the solid residue left after extraction, the solids of the extract computed from an aliquot evaporated to dryness and weighed, and the dry weight of the substances removed from the extract in solution in chloroform.

SUGARS.—In estimating reducing sugars, 50 cc. of the extract was measured off, cleared with basic lead acetate solution, and delead with potassium oxalate solution. The volume was then adjusted, and the reducing sugars of 50 cc. were computed as glucose by the Bertrand volumetric method, and calculated for the entire sample.

The non-reducing sugars were computed as sucrose. From the solution previously cleared and delead, 50 cc. was measured off and 5 cc. of HCl added. This mixture was heated for 1 hour on the hot-water bath and then set aside for 48 hours. It was then neutralized with sodium hydroxide and sodium carbonate, and the volume adjusted to 100 cc. The total reducing sugar was computed as invert sugar by the Bertrand volumetric method. The reducing-sugar content before hydrolysis was also computed as invert sugar. The difference between these multiplied by 0.95 gave the sucrose, which was computed for the entire sample.

POLYSACCHARIDES.—For the determination of the total polysaccharides, a small sample of the dry solid residue was weighed off into a Kjeldahl flask, and to this were added 200 cc. of water and 12.5 cc. of concentrated HCl. This mixture was boiled under a reflux condenser for 3 hours. The mixture was then cooled and neutralized with sodium hydroxide and sodium carbonate, cleared and delead, and its volume adjusted. The reducing sugar was estimated as glucose by the Bertrand method, and the weight of the polysaccharides was calculated by multiplying this result by 0.9. The weight of the polysaccharides was then calculated for the entire sample.

The starch was estimated from the results of saliva digestion, the method employed by CLEMENTS (3) in determining the starch content of leaves. To secure gelatinization of the starch, a small amount of the dry residue left after extraction was weighed off, put in a pyrex beaker, and moistened with water. After a few minutes 50 cc. of water was added. The mixture was then boiled for a moment over a gas burner, the inside of the beaker washed down, and the beaker, covered with a watch glass, transferred to the hot-water bath for 1 hour. The contents of the beaker were then cooled to approximately 37° C., and 10 cc. of filtered saliva was added. The mixture was incubated over night at 37° C. A check sample of the material was treated in the same way, and samples of this were tested with iodine and examined microscopically to make sure that the hydrolysis of the starch was complete.

After the starch digestion was complete, the mixture was cleared, delead, and its volume adjusted. From this, 100 cc. was taken and acidified to the extent of 2.5 per cent with sulphuric acid and heated on a boiling hot-water bath for 1.5 hours to hydrolyze to glucose the maltose resulting from the starch digestion. The solution was then neutralized with sodium hydroxide and sodium carbonate and its volume adjusted. The glucose was estimated by the Bertrand method and the starch calculated by multiplying this result by 0.9.

The hemicelluloses were determined by taking the difference between the total polysaccharides and the starch.

TOTAL CARBOHYDRATES.—The total carbohydrate content was determined by adding together the reducing sugars, sucrose, and total polysaccharides.

NITROGEN COMPOUNDS.—The total nitrogen of the extract was determined by the Gunning method modified to include the nitrogen of the nitrates. The organic nitrogen of the extract was determined by the Gunning method. For these determinations 50 cc. samples of the extract were dried in Kjeldahl flasks on the hot-water bath. The amino-acid nitrogen of the extract was determined by the Van Slyke method. The nitrogen of the solid residue was determined by the Gunning method. The weight of the protein was obtained by multiplying this result by 6.25. The total nitrogen of the sample was found by taking the sum of the total nitrogen of the extract and that of the solid residue.

EXPERIMENT III

In this experiment the respiratory carbon dioxide was determined by the method of double titration, and the rates of respiration in the treated and untreated seeds after sprouting were thus compared. The air was drawn into the respiratory chamber through a series of five wash bottles, the first four containing sodium hydroxide solution and the last barium hydroxide; and from the respiratory chamber through the gas absorption bottle (Milligan) containing 250 cc. of normal sodium hydroxide solution for the absorption of the respiratory carbon dioxide; then through a wash bottle containing barium hydroxide, and a safety flask connected with the aspirator by means of which the air was drawn through the apparatus at a uniform rate.

Two sets of seeds were used. In set no. 1 the seeds were untreated (presoaked, sterilized, and washed); in set no. 2 the seeds were presoaked, sterilized, washed, and soaked in sterilized distilled water with aeration in the usual way 3 days. The seeds of each set were sprouted in a germinator until the sprouts were approximately 2 cm. long before being put in the respiration chamber. Many of the seeds of set no. 2 did not sprout. Twenty-five sprouted seeds were used in each of the tests. These respiration tests were carried out at a temperature of approximately 25° C. They were made in duplicate, and were continued exactly 10 hours. At the end of this period the solution in the gas absorption bottle was titrated with normal and 0.1 normal sulphuric acid with phenolphthalein indicator, and then with 0.1 normal sulphuric acid with methyl orange indicator. After correction with blank tests on the normal sodium hydroxide, the second

titration gave the measurement of the respiratory carbon dioxide. As a result of the first titration, the carbon dioxide is held in the form of NaHCO_3 . The carbon dioxide is set free in the second titration.

EXPERIMENT IV

In this experiment the effect on catalase activity of soaking seeds in distilled water both with and without aeration was investigated. For washing and soaking the seeds, distilled water sterilized in the autoclave and cooled in the ice-box was used. The seed treatment consisted of presoaking in distilled water 10 minutes, sterilizing with 0.25 per cent uspulun solution 15 minutes, washing with distilled water, and soaking in distilled water. After soaking the seeds were dried in the draft of an electric fan for 48 hours to the air-dry state (12). The untreated seeds were used in their original dry state, but were exposed to the draft of the electric fan with the soaked seeds in order to bring them as nearly as possible to the same degree of dryness.

The method employed by SHULL and DAVIS (17) was followed in the catalase tests, Oakland "dioxogen" being used. The tests were run in triplicate. Not less than 20 seeds were used in preparing the material for each test. The seed coats, hypocotyls, and plumules were removed. The cotyledons were finely ground in a glass mortar, and this material was sifted through a 100-mesh sieve. Of this material, 0.2 gm. was used in each test. In order to neutralize acids, 0.5 gm. of pure calcium carbonate was mixed with 10 cc. of the dioxogen in the funnel of the catalase apparatus. The percentage of dry matter in the pulverized material was determined, and the oxygen liberated by the catalase was calculated on the basis of the dry weight.

In experiment IVa, three sets of seeds were used. In set no. 1 the seeds were untreated; in sets 2 and 3 the soaking with aeration was continued 2 and 5 days respectively, the water being changed four times daily during the soaking. Each seed chamber was placed in a water bath and the temperature thus kept at 12° – 14° C. The apparatus and method employed in soaking the seeds with aeration were those used in the preceding experiments (fig. 1). In experiment IVb also three sets of seeds were used. In set no. 1 the seeds were untreated; in sets 2 and 3 the soaking without aeration was con-

tinued 2 and 5 days respectively. The seeds were soaked in Erlenmeyer flasks of 500 cc. capacity, plugged with cotton, and previously sterilized in the autoclave. Precautions were taken to prevent contamination of the seeds with bacteria, and no indications of bacterial growth during soaking were seen. The water was not changed during the period of soaking. The seeds were kept in the ice-box at a temperature of 12°-14° C. during the soaking period.

Results

EXPERIMENT I.—The results of this experiment show a progressive decrease in the rate of germination resulting from the seed treatment. There is a still greater reduction in the number of mature plants pro-

TABLE I

INFLUENCE OF SEED TREATMENT ON GERMINATION (APPEARANCE OF SEEDLINGS AT SURFACE OF SOIL), NUMBER OF MATURE PLANTS PRODUCED, AVERAGE WEIGHT, AND NUMBER OF DAYS REQUIRED TO REACH MATURITY

PERIOD OF TREATMENT	NO. OF SEEDS	NO. GERMINATED	PERCENTAGE GERMINATION	NO. OF MATURE PLANTS	PERCENTAGE MATURED	AV. WEIGHT OF PLANTS (GM.)	DAYS TO REACH MATURITY
Untreated.....	100	95	95.00	91	91.00	26.26	37
8 hours.....	120	97	80.83	84	70.00	24.50	38
2 days.....	120	76	63.33	71	59.17	22.88	38
5 days.....	140	46	32.86	31	22.14	17.48	43
7 days.....	140	7	5.00	3	2.14	16.67	48
9 days.....	140	1	0.71	1	0.71	14.50	46

duced, since a considerable number of them from treated seeds were unable to survive until they reached maturity. There is also a progressive decrease in the average weight of the tops, and an increase in the time required for the plants to reach maturity with the lengthening of the period of seed treatment. The results are shown in table I.

Figure 2 shows the effect of the seed treatment on growth of the plants. The two plants were at the same stage of development. They were grown in flower pots, and the roots were removed from the soil so as to secure the entire root system. The plant on the left is typical of those grown from untreated seed, that on the right of those grown from seed treated 5 days. Flower buds may be seen on each of them. The plant from untreated seed reached this stage in

44 days, that from treated seed in 50 days. The plants from treated seeds showed marked reduction in growth resulting from the seed treatment. Both the shoot and the root system showed this reduction in growth, apparently in about the same proportion.

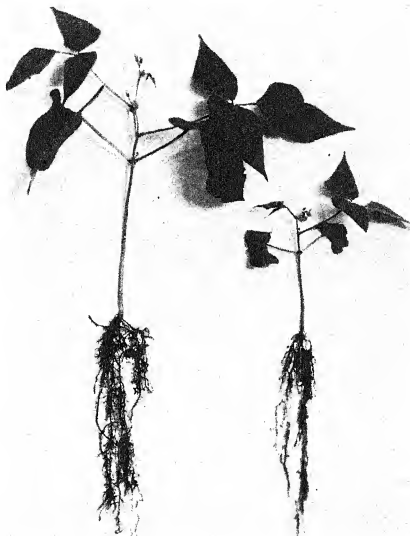


FIG. 2.—Effects on subsequent growth of soaking bean seeds in distilled water with aeration: left, plant from untreated seed, 44 days after planting; right, plant from seed soaked with aeration 5 days, 50 days after planting.

In the plants from untreated seeds the average diameter of the internode of the stem immediately above the first pair of leaves was 3.497 mm., the average thickness of the xylem was 0.474 mm., and the average thickness of the phloem was 0.07953 mm. The ratio of the average thickness of the phloem to the xylem was 0.168. In the

plants produced from seeds treated 5 days the average diameter of the same internode of the stem was 3.377 mm., the average thickness of the xylem 0.452 mm., and the average thickness of the phloem 0.1155 mm. The ratio of the phloem to the xylem was 0.255. It will be noted that there is a marked increase in the average thickness of the phloem and in the ratio of the phloem to the xylem in the plants from the treated seeds.

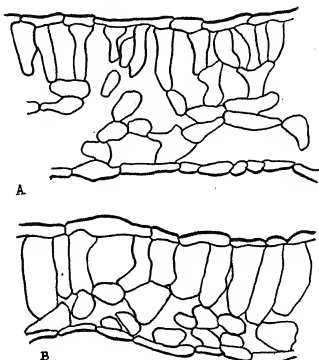


FIG. 3.—Cross-sections of leaves: *A*, section of typical leaf of plant from untreated seed; *B*, section of typical leaf of plant from seed soaked with aeration 5 days.

The difference in leaf structure in the plants from untreated seeds and those from seeds treated 5 days is shown in figure 3. Figure 3 *A* shows the cross-section of a leaf of a mature plant from untreated seed, figure 3 *B* shows that of a mature plant from seed treated 5 days. The sections shown in the figure are representative of the leaf structure found generally in the two sets of plants, the sections being drawn to the same scale. There is a marked decrease in thickness and increase in compactness of structure in the leaf of the plant from the treated seed.

EXPERIMENT II.—This experiment deals with the influence of soaking seeds in distilled water with aeration on germination, num-

ber of mature plants produced, average weight, time required to reach maturity, percentage of dry matter and water content, abundance of carbohydrates and nitrogenous compounds; and the influence on growth of the presence of a calcium salt in very dilute solution in the water in which the seeds were soaked.

Because of the shorter days and lower light intensity of late autumn and winter, there was but little branching in these plants, and their size and weight were much less than in the plants in experiment I. The plants grew and matured normally, however, and the results

TABLE II

GERMINATION OF SEEDS (APPEARANCE AT SURFACE OF SOIL), NUMBER OF MATURE PLANTS PRODUCED, AVERAGE WEIGHT, AND NUMBER OF DAYS REQUIRED TO REACH MATURITY:

1, SEEDS UNTREATED

2, SEEDS SOAKED WITH AERATION 5 DAYS IN DISTILLED WATER

3, SEEDS SOAKED WITH AERATION 5 DAYS IN DISTILLED WATER CONTAINING CALCIUM NITRATE (APPROXIMATELY 900×10^{-6} GM. NORMAL PER LITER)

No.	No. OF SEEDS	No. GERMINATED	PERCENTAGE GERMINATION	No. OF MATURE PLANTS	PERCENTAGE MATURED	AV. WEIGHT OF PLANTS (GM.)	DAYS TO REACH MATURITY
1.....	100	90	90.00	80	80.00	2.80	41
2.....	140	98	70.00	54	38.57	1.86	48
3.....	105	71	67.62	48	45.71	2.08	48

indicate similar effects from seed soaking in reduction of growth. There is a decrease in germination and a greater decrease in the number of plants able to reach maturity, a marked reduction in average weight, and an increase in the time required to reach maturity. The results obtained from the addition of calcium nitrate to the water indicate that in general this addition did not greatly modify the effects of the soaking. The results are shown in tables II and III.

The data given in table III indicate that there is an increase in the percentage of dry matter in the plants produced from seeds soaked with aeration 5 days in distilled water, in comparison with the plants from untreated seeds.

One of the most striking results of the predetermining influence of soaking the seeds is the relative increase in the carbohydrates in

the plants produced from the treated seeds, in comparison with those from untreated seeds. This increase is seen in the reducing

TABLE III
PERCENTAGE OF DRY MATTER AND MOISTURE

SEED TREATMENT	PERCENTAGE	
	DRY MATTER	MOISTURE
Untreated.....	16.61	83.39
5 days in H ₂ O.....	19.05	80.95

TABLE IV
GLUCOSE, SUCROSE, AND TOTAL SUGARS EXPRESSED AS PERCENTAGES
ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS					
	GLUCOSE		SUCROSE		TOTAL SUGARS	
	TOTAL	DRY	TOTAL	DRY	TOTAL	DRY
Untreated.....	0.299	1.800	0.309	1.921	0.608	3.721
5 days in H ₂ O.....	0.443	2.320	0.344	1.804	0.787	4.124

TABLE V
STARCH, HEMICELLULOSES, AND TOTAL POLYSACCHARIDES EXPRESSED AS
PERCENTAGES ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS					
	STARCH		HEMICELLULOSES		TOTAL POLYSACCHARIDES	
	TOTAL	DRY	TOTAL	DRY	TOTAL	DRY
Untreated.....	0.672	3.857	1.212	7.481	1.883	11.338
5 days in H ₂ O.....	1.635	8.587	1.330	6.985	2.965	15.572

sugars and total sugars, starch, and total polysaccharides. These results are shown in tables IV-VII. The reducing sugars are computed as glucose and the non-reducing sugars as sucrose.

The data given in tables VIII and IX indicate that the predetermining influence of soaking the seeds resulted in a decrease in total nitrogen, organic nitrogen, amino-acid nitrogen, and proteins.

TABLE VI
TOTAL CARBOHYDRATES EXPRESSED AS PERCENTAGES
ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASES	
	TOTAL	DRY
Untreated.....	2.491	15.059
5 days in H ₂ O.....	3.752	19.699

TABLE VII
CARBOHYDRATES EXPRESSED AS PERCENTAGES OF WEIGHTS OF THE CARBOHYDRATES
FOUND IN PLANTS FROM UNTREATED SEEDS; BASIS OF EQUAL WEIGHTS OF
WET AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASES											
	GLUCOSE		SUCROSE		TOTAL SUGARS		STARCH		HEMICEL- LULOSES		TOTAL CARBO- HYDRATES	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None.....	100	100	100	100	100	100	100	100	100	100	100	100
5 days in H ₂ O....	148	129	111	94	129	111	243	223	110	93	151	131

TABLE VIII
TOTAL NITROGEN, ORGANIC NITROGEN, AMINO-ACID NITROGEN, AND PROTEINS
EXPRESSED AS PERCENTAGES OF WEIGHTS OF WET
AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS							
	TOTAL NITROGEN		ORGANIC NITROGEN		AMINO-ACID NITROGEN		PROTEINS	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None.....	0.623	3.749	0.619	3.729	0.021	0.126	2.745	16.531
5 days in H ₂ O....	0.543	2.851	0.540	2.836	0.017	0.088	2.545	13.303

EXPERIMENT III.—This experiment, designed to measure the effects of seed soaking upon subsequent respiration, presents a quantitative determination of the carbon dioxide given off in respiration by treated and untreated seeds after sprouting. As is shown by table X, there was no decrease in respiratory carbon dioxide from sprouted seeds in the case of the soaked seeds in comparison with those not soaked. On the contrary, the results showed an increase.

TABLE IX

TOTAL NITROGEN, ORGANIC NITROGEN, AMINO-ACID NITROGEN, AND PROTEINS EXPRESSED AS PERCENTAGES OF WEIGHTS OF THESE FOUND IN PLANTS FROM UNTREATED SEEDS; BASIS OF EQUAL WEIGHTS OF WET AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS							
	TOTAL NITROGEN		ORGANIC NITROGEN		AMINO-ACID NITROGEN		PROTEINS	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None.....	100	100	100	100	100	100	100	100
5 days in H ₂ O...	87	76	87	76	80	70	93	81

TABLE X

WEIGHT OF RESPIRATORY CARBON DIOXIDE GIVEN OFF PER
100 GM. OF DRY MATTER IN 10 HOURS

	SEED TREATMENT	
	UNTREATED	3 DAYS IN H ₂ O
Grams of CO ₂	1.191	1.568

EXPERIMENT IV.—It was thought that soaking the seeds might influence their catalase activity. The data given in table XI indicate that in the case of seeds soaked with aeration there is a small decrease in catalase activity in the seeds soaked 2 days, but an increase in those soaked 5 days. On the other hand, table XII shows that in the case of the seeds soaked without aeration there is a considerable decrease in catalase activity resulting from soaking for 2 days, and a further decrease from a soaking period of 5 days. The

volume of oxygen has been computed for 1 gm. of air-dry material and 1 gm. of dry matter.

TABLE XI

VOLUME OF OXYGEN LIBERATED BY CATALASE ACTION AND PERCENTAGE OF OXYGEN ON BASIS OF THAT LIBERATED BY UNTREATED SEEDS; SEEDS SOAKED WITH AERATION; VOLUMES CORRECTED TO 0° C. AND 760 MM. PRESSURE; 10-MINUTE RUNS

SEED TREATMENT	0.2 GM. AIR- DRY MATERIAL (CC.)	1.0 GM. AIR- DRY MATERIAL (CC.)	1.0 GM. DRY MATTER (CC.)	PERCENTAGE OXYGEN
Untreated.....	19.97	99.85	113.04	100.00
2 days in H ₂ O...	19.04	95.20	108.02	95.56
5 days in H ₂ O...	22.49	112.45	129.47	114.53

TABLE XII

VOLUME OF OXYGEN LIBERATED BY CATALASE ACTION AND PERCENTAGE OXYGEN ON BASIS OF THAT LIBERATED BY UNTREATED SEEDS; SEEDS SOAKED WITHOUT AERATION; VOLUMES CORRECTED TO 0° C. AND 760 MM. PRESSURE; 10-MINUTE RUNS

SEED TREATMENT	0.2 GM. AIR- DRY MATERIAL (CC.)	1.0 GM. AIR- DRY MATERIAL (CC.)	1.0 GM. DRY MATTER (CC.)	PERCENTAGE OXYGEN
Untreated.....	23.02	115.10	125.89	100.00
2 days in H ₂ O...	20.32	101.60	111.87	88.86
5 days in H ₂ O...	19.27	96.35	106.43	84.54

Discussion

KIDD and WEST (4) found no decrease in the rate of germination in bean seeds soaked 6 hours, and but little decrease from a treatment of 24 hours; but practically complete failure of germination resulting from treatment for 72 hours. Their report shows no provision for aeration of the seeds or elimination of bacteria. TILFORD, ABLE, and HIBBARD (19), who sterilized the seeds to eliminate bacteria and aerated them during the soaking treatment, came to the conclusion that bean seeds may be expected to germinate as well under water as in the soil, provided there are sufficient oxygen for respiration, removal of the carbon dioxide and other by-products, and elimination of the bacteria. RHINE (15) found that wheat soaked in sterilized distilled water 22 days gave a germination test of only 2

per cent, while that soaked 29 days showed a complete failure of germination. BARTON (1) concluded that "the capacity for germination is not destroyed by long continued soaking in sterile media."

In this investigation, the seeds after soaking were planted at a depth of approximately 1 inch in the soil. Germination was considered to have taken place when the seedlings appeared at the surface of the soil. The results of the germination tests are shown in table I. It will be noted that soaking for 8 hours decreased the rate of germination from 95 to 80.83 per cent, and that there was a progressive decrease in the rate of germination as the length of the period of seed treatment was increased. The rate of germination was reduced to 5 per cent by 7 days' treatment. It should be borne in mind that in these experiments the seeds were freed from bacteria by sterilization, and that they were aerated during the period of soaking. The seeds were subjected to the action of a large volume of water, since the water used in soaking was completely changed four times per day. It is evident that soaking in distilled water had an injurious effect on germination, and that germination practically ceased when the seeds had been subjected to soaking for 7-9 days. These results seem to differ radically from those obtained by KIDD and WEST, TILFORD, ABLE, and HIBBARD, and BARTON. In the work of these investigators, the seeds were subjected to the action of comparatively small volumes of water, since the water was not changed during the soaking period. Also these workers did not germinate the seeds in the soil, while in this investigation the seeds were planted in the soil and the seedlings had to grow sufficiently to appear at the surface of the soil before the seeds were considered to have germinated. Table II shows the injurious effect on germination of a prolonged period of soaking. Data given in tables I and II show the further reduction in the number of plants from soaked seeds which were able to reach maturity, a considerable number of plants from treated seed dying before the mature stage was reached.

The plants photographed in figure 2 show the deleterious influence of seed soaking on subsequent growth of the plant. The reduction in growth in the shoot and root system is in about the same proportion. The increase in time required for the plants from treated seeds to reach the blooming stage also indicates the injurious effect of the

seed treatment on the rate of growth. This is further shown in the progressive decrease in the average weight of the plants (tables I, II).

TRUE (20) studied the harmful action of distilled water and the significance of calcium for higher green plants, using the electrical conductivity method. He found that the samples of distilled water showing the highest electrical resistance were in general more harmful to lupine roots than water containing larger quantities of electrolytes. He also found that these samples of water withdrew electrolytes from the tissues of the roots. This was taken to be the probable mechanism causing the harmful action. According to TRUE, the distilled water seems to withdraw material required for maintenance of the efficient action of the protoplasmic limiting membranes, with the result that cell permeability is increased and a further dissociation of the electrolytes from their points of combination in the proteins and other chemical structures of the cells ensues; but when a calcium salt is added to the distilled water in sufficient concentration to make it osmotically equal to tap water, this dissociating power of the water is largely undeveloped, the leaching is checked, and the chemical integrity of the cells is maintained. TRUE and BARTLETT (21) came to the conclusion that there are equilibrium concentrations for calcium and nitrate ions and for magnesium and nitrate ions at which leaching is largely overcome, and below which roots would leach ions into either solution or into both solutions mixed in various proportions. It was shown that calcium ions differ from magnesium ions in being harmless in concentrations that prove fatal in the case of magnesium. In no concentration of a calcium salt that was tried, the strongest being approximately 900×10^{-6} gm. normal per liter, was there any indication of injury.

SCARTH (16) investigated the toxic action of distilled water on *Spirogyra* and its antagonism by cations. He came to the conclusion that distilled water free from metal ions but exposed to the air is highly toxic to this plant, that the positive toxic factor is the H-ion concentration due to the dissolved CO_2 , and that cations are antagonistic to this toxic action according to their valencies, being limited also by their own toxicity.

In this investigation, experiment II was modified to determine

whether the addition of a calcium salt to the distilled water in which the seeds were soaked would decrease or modify the deleterious influence of soaking on subsequent growth of the plants. The highest concentration of calcium nitrate employed by TRUE, approximately 900×10^{-6} gm. normal per liter, was used. The data in table II lead to the conclusion that the injurious effects of soaking on the rate of germination and on subsequent growth were not greatly modified by the addition of the calcium salt. Table III shows that one of the results of soaking is an increase of dry matter or decrease in moisture in the mature plants produced, in comparison with plants produced from untreated seeds.

One of the very evident effects of the predetermining influence of seed soaking is the higher percentage of carbohydrates in the mature plants from the treated seeds. This is shown in tables IV-VII. The percentage of reducing sugar is higher in the plants from the treated seeds, and the increase in starch resulting from the seed treatment is very striking.

The data in tables VIII and IX lead to the conclusion that another effect of the predetermining influence of seed soaking is a relative decrease in total nitrogen, organic nitrogen, amino-acid nitrogen, and proteins. While this decrease in nitrogen compounds is not so pronounced as the increase in carbohydrates, yet it seems to be very evident.

A correlation between the predetermining influence of this seed treatment and the internal metabolism of the plants produced from the treated seeds may be seen in the carbohydrate-nitrogen relations of the plants, and the moisture and dry matter content. The plants from treated seeds, which were higher in carbohydrates and lower in nitrogen and nitrogen compounds, were lower in moisture content and higher in dry matter. On the other hand, the plants from untreated seeds, which were lower in carbohydrates and higher in nitrogen and nitrogen compounds, were higher in moisture and lower in dry matter (10).

It seems probable that the greater accumulation of carbohydrates in the plants from the treated seeds is due to a decrease in the ability of these plants to form amino acids, proteins, and related nitrogen compounds from the carbohydrates, resulting from soaking the

seeds. The reduction in growth in these plants may be a result of a reduction in the nitrogen compounds. A comparison of the two plants shown in figure 2 leads to the conclusion that the plant from the treated seed has as extensive a root system in proportion to the shoot as has that from the untreated seed. Evidently the decrease in the nitrogen compounds and water in the plants from the treated seeds is not due to reduced absorption caused by a poorly developed root system.

The data in table X indicate that there was no decrease in the production of respiratory carbon dioxide by the sprouted seeds resulting from soaking. Some of the soaked seeds did not sprout, of course, and only sprouted seeds were used in the test. This result indicates that the soaking treatment does not injure the respiratory enzymes or mechanism of the seeds, and that the reduction in growth is not due to a decrease in the rate of respiration. In fact, the production of respiratory carbon dioxide was somewhat higher in the sprouted seeds previously soaked than in those not previously soaked. Probably the higher water content of the former was more favorable to the respiratory process. The water content of the sprouted seeds previously soaked, at the stage at which the test for respiratory carbon dioxide was made, was 63.9 per cent; while that of the seeds sprouted without being previously soaked, at the same stage, was 53.7 per cent. MAXIMOV (11) gives the results obtained by KOLKWITZ in connection with the relation of moisture to respiration in barley. He found that 1 kg. of barley grain containing 10-12 per cent of moisture exhaled in 24 hours 0.0003-0.0004 gm. of carbon dioxide; with 14-15 per cent moisture, 0.0013-0.0015 gm. of carbon dioxide; and that with a further increase in water content, respiration increased rapidly, reaching 2.0 gm. of carbon dioxide in 24 hours, when the grain was almost completely swollen.

Table XI indicates that soaking 2 days in distilled water with aeration caused a decrease of 4.44 per cent in the oxygen liberated from hydrogen peroxide by the catalase activity of the seeds, while soaking with aeration 5 days caused an increase of 14.53 per cent. On the other hand, the data in table XII show that soaking without aeration resulted in a continuous decrease in catalase action, amounting to 11.14 per cent in the seeds soaked 2 days and 15.46 per cent in

those soaked 5 days. The latter result is in harmony with that obtained by RHINE (15). In using wheat soaked (without aeration) in distilled water previously boiled and cooled, she found that there was a continuous decrease in catalase activity resulting from soaking, the catalase action practically ceasing after a soaking period of 29 days. In the case of soaking with aeration, the seeds passed through the early stages of germination during the soaking treatment. There was the usual development of catalase activity in connection with germination, amounting to an increase of 14.53 per cent when the seeds had been soaked 5 days.

Summary

1. Soaking seeds of dwarf bean in distilled water with aeration resulted in a considerable decrease in the rate of germination (appearance of the seedlings at the surface of the soil) for periods of soaking as short as 8 hours. Practically a complete failure of germination resulted from soaking periods of 7 to 9 days.

2. The soaking treatment resulted in a still further reduction in the number of mature plants produced, as some of the seedlings from the treated seeds were unable to reach maturity.

3. The soaking treatment resulted in a progressive decrease in growth and weight of the plants produced and an increase in the time required for the plants to reach maturity.

4. The seed treatment resulted in general in a modification of the leaf structure, the leaf blades of plants from treated seeds generally being thinner and more compact in structure than those of plants from untreated seeds.

5. The greater average thickness of the phloem and higher ratio of the phloem to the xylem in the stems of the plants from soaked seeds is probably correlated with the relatively higher carbohydrate and lower nitrogen content of these plants.

6. The addition of calcium nitrate in very dilute solution to the water in which the seeds were soaked did not prevent the deleterious influence of soaking on germination and growth.

7. Soaking the seeds resulted in a marked relative increase in reducing sugars, starch and total carbohydrates, and solid matter, and in a relative decrease in total nitrogen, organic nitrogen, amino-acid

nitrogen, and proteins in the mature plants produced from the soaked seeds. A correlation may be seen in the decreased nitrogen associated with decreased moisture and increased carbohydrates.

8. It seems probable that the greater relative accumulation of carbohydrates and deficiency of nitrogen compounds in the plants from the soaked seeds are due to a decrease in the ability of these plants to form the nitrogen compounds from the carbohydrates. The reduction in growth is probably due to the reduction in nitrogen compounds.

9. There was an increase in the rate of production of respiratory carbon dioxide in the sprouted seeds soaked before sprouting in comparison with those sprouted without previous soaking. This increase was probably due to the fact that the treated seeds contained considerably more water than untreated ones. The higher water content was favorable to a more rapid rate of respiration. The reduction in growth, of course, was not connected with any decrease in the rate of respiration.

10. Soaking the seeds without aeration resulted in a regular decrease in the catalase activity. Soaking with aeration resulted at first in a small decrease in catalase activity, but this was soon followed by a considerable increase. This modification of the result is evidently due to the fact that the seeds when soaked with aeration pass through the early stages of germination, and consequently there is an increase in catalase activity characteristic of seed germination.

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PARALLELISM OF PRECIPITATION REACTIONS AND BREEDING RESULTS IN THE GENUS *IRIS*¹

I. PRELIMINARY STUDY AND CORRELATION WITH OTHER EVIDENCE

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(WITH ONE FIGURE)

Introduction

A preliminary study has been made of the precipitation reactions between some of the more distinct subgroups of the genus *Iris*. The purpose has been to determine whether a correlation exists between the taxonomy of the genus and the precipitation reactions of its species. Evidence from breeding experiments, from the few known chromosome numbers, and from chemical analyses is discussed also in this connection. Further investigations will include other groups of the genus. By placing the chief emphasis on the reactions within one genus, this study differs somewhat from the work of KOSTOFF (14) and CHESTER (5).

The classification of *Iris* which is generally followed is that proposed by DYKES (8). It is used, with a few changes in order, by DIELS in his treatment of Iridaceae, in ENGLER and PRANTL (10). As a result of their recent discoveries in Louisiana, SMALL and

¹ At the suggestion of Professor G. K. K. LINK, we have used "precipitation reaction" in place of "normal precipitin reaction," as proposed by others. We quote from his letter:

"*Precipitin reaction* is a term used in serology and immunology for a specific type of precipitation reaction. It involves the reaction of the blood sera of animals that have been modified *in vivo* by the presence of foreign substances that contain or consist of proteins or protein combinations with other substances. These substances, whether particulate or in solution, are designated as antigens, because they engender specific antibodies in blood sera. If the presence of such antibodies is detectable *in vitro* by a reaction involving precipitation of a combination of the antibody and of a test antigen which is in solution, the antibody is designated as a *precipitin* and the reaction a *precipitin reaction*. Antigens may get into the blood stream spontaneously, or by artificial means, such as injection. Precipitins engendered by anti-

ALEXANDER (21) have adopted a new partial classification, one, however, which they themselves term "incomplete and necessarily tentative."

The approximately 160 species of *Iris* recognized by DYKES are placed in two major groups characterized by different habit, the rhizomatous and the non-rhizomatous. Rhizomatous irises fall into seven sections, and non-rhizomatous (bulbous) into three sections, marked not only by taxonomic differences but also by differences in geographic distribution. In addition, there are two other small sections, one of two species, characterized neither by bulb nor rhizome, said by DYKES (9) to resemble *Hemerocallis*, and one further species, *Iris sisyrinchium* L., which has a corm and is more nearly related to *Moraea* than to *Iris* (9).

Of the 12 sections of *Iris*, the most wide-spread is the Apogon (beardless) section, members of which are found in Europe, North Africa, and America. This great range is accompanied by marked diversity of habitat, unlike other sections of the genus. It is in this group also that there is found the greatest range of chromosome numbers, from $n=12$, in *Iris pseudacorus* L. and others, to $n=ca. 56$ in *I. virginica* Dickson (11, 12). The large section Pogoniris (bearded) is confined to a smaller range throughout Europe, North Africa, and the Near and Middle East. Its members display marked

gens not artificially introduced are called *normal precipitins*, and the test reaction a *normal precipitin reaction*.

The plant reactions reported by KOSTOFF, CHESTER, and in your paper consequently are neither normal precipitin reactions nor precipitin reactions following injections of antigens. Unfortunately, the latter have been referred to as "acquired precipitin reactions" in the botanical literature.

Obviously, in order to avoid confusion and misunderstanding, until more is known about the nature of the reactions involved in the direct tests between plant extracts and in the tests using blood antisera, it is advisable to avoid the serological and immunological terms which now have definite meanings. I had decided before receipt of your paper to suggest in a brief paper that the non-committal term "precipitation reaction" be used for the interesting reactions of plant extracts which do not involve blood sera, until it is demonstrated whether these and the precipitin reactions are fundamentally identical or dissimilar."

While the precipitin test is one involving antigens and antibodies engendered in blood sera by antigens, the precipitation reaction as referred to in this paper simply indicates the formation of precipitates in which antigens and blood sera are not involved.

uniformity in habitat, and the known chromosome numbers fall into distinct groups, correlated with the taxonomic subdivisions, varying as follows: $n=12$, $n=20$, $n=22$, and $n=24$ (11, 12). The *Oncocyclus*, *Regelia*, and *Pseudoregelia* sections are confined to even smaller ranges in the Near East and Central Asia. They likewise display great uniformity in chromosome numbers; in *Oncocyclus* $2n=20$, and in *Regelia* $2n=44$, with few exceptions (11, 12). Each of the three bulbous sections, *Juno*, *Reticulata*, and *Xiphium*, is found in a relatively small geographic range; with but one exception all the species require a warm, dry habitat; the few known chromosome numbers range from $n=9$ in *I. caucasica* Hoff. to $n=21$ in *I. xiphoides* Ehrh. (11, 12). The small *Evansia* (crested) section has representatives only in southeastern Asia and the United States, and the chromosome numbers vary from $n=12$ in *I. cristata* Ait. to $n=18$ in *I. japonica* Thunb. (11, 12). It might be noted that SMALL and ALEXANDER (21) remove *I. verna* L. and *I. cristata* to another genus, *Neubeckia* Alef.

Material and methods

The species, varieties, and hybrids used in this study represent six of the twelve sections of the genus, and include eight of the fifteen subsections of the *Apogon* section. They were secured from the *Iris* collection at Connecticut College and from Mrs. LOUISE W. KELLOGG of West Hartford, Connecticut, who has generously provided us with material on two occasions. Wherever possible, only species and varieties were used, but certain horticultural hybrids of known parentage have been included, as well as a beardless Japanese iris of unknown parentage. So far as is known, all material was correctly identified; but the possibility of misidentification must not be left out of consideration, especially as most of the material was collected after the blooming period was over. The following plants were used: a recognized albino form of *I. orientalis* Thunb., called Snow Queen, *I. forrestii* Dykes, *I. prismatica* Pursh, *I. unguicularis* Poir., *I. graminea* L., *I. halophila* Pall., *I. ochroleuca* L., a probable descendant of *I. kaempferi* Sieb. called Albatross, *I. pseudacorus* L., *I. pseudacorus* var. *bastardi* Spach., *I. fulva* Ker-Gawl., *I. hexagona* Walt., *I. foliosa* Mackenzie and Bush, two hybrids of *fulva* \times *foliosa* parentage called Fulvala and Dorothea K. Williamson, *I. chryso-*

phoenicia Small, *I. hyacinthiana* Farrer, *I. tricuspis* Dickson (probably merely a variety of one of the Tripetalae), *I. setosa* Pall. var. *canadensis* Foster, *I. verna* L., *I. vinicolor* Small, *I. dichotoma* Pall., *I. tectorum* Maxim., *I. gracilipes* Gray, *I. cristata* Ait., *I. stolonifera* Maxim. var. *vaga* Foster, *I. hoogiana* Dykes, *I. kochii* Kerner, Marjory Tinley (a garden hybrid between two forms of *I. pallida* Lam.), and *I. bucharica* Foster.

In general, the technique used in this study was that of KOSTOFF (14) and CHESTER (5), but certain modifications were found necessary. Leaves of the plants to be studied were collected, weighed, and washed in tap and distilled water. After being cut into pieces as small as possible, they were ground in unglazed mortars; the paste thus obtained was placed in an Erlenmeyer flask and to it was added distilled water to the amount of twice the weight of the green leaves. The flasks were then placed in a cold chamber at 3°-5° C. After 24 hours the extracts were filtered into test-tubes (one filtering usually being sufficient) and again placed in the cold chamber. Some were covered with a thick layer of toluene for preservation over a longer period of time. The extracts were mostly crystal clear and colorless, although some had a slight yellowish tinge, and that of *I. pseudacorus* was opalescent.

An attempt to use extracts from dried material as advocated by CHESTER (5) proved unsatisfactory, since no results were obtained from such extracts against each other; on the other hand, in some instances these extracts from dried material gave positive reactions when tested against extracts from green material.

In making the tests, small tubes of several sizes were used, varying from 3 to 8 mm. in diameter and 50-70 mm. in length. It was found that in most cases a 6-mm. tube gave the best results. The heavier liquid was placed in a tube, and the lighter liquid carefully placed above it by means of a fine pipette drawn out to a tip opening approximately 0.5 mm. in diameter. Observations were then made over a period of 45 minutes. In a positive reaction, a ring of precipitate of varying intensity was formed between the two liquids. This ring gradually widened until, in some instances, both liquids became cloudy. KOSTOFF (14, p. 47) noted that in many cases where precipitation was very weak, the precipitate completely disappeared after a time.

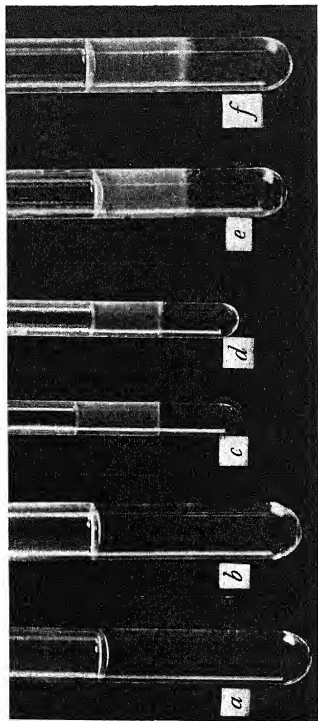


FIG. 1.—*a*, precipitation test between extracts of *I. foliosa* and *I. orientalis*, showing no reaction (—); *b*, similar test between extracts of *I. foliosa* and *I. orientalis*, showing a trace (±); *c*, similar test between extracts of *I. foliosa* and *I. pseudacorus*, showing a (+) reaction; *d*, similar test between extracts of *I. tectorum* and *I. pseudacorus*, showing a (+ + +) reaction; *e*, *f*, later stages of *c* and *d* respectively.

No instance of such a disappearance was noted in any of our tests, although tubes were sometimes allowed to remain for a long period. He noted (15, p. 99) also that different individuals of the same species may give reactions of differing intensities. In some cases our tests confirm this point. No lytic rings appeared. The results of the tests are given in table I, all readings being minimal, and blank spaces in the table indicating that no test was made. The small amount of material available in some cases made a complete series of tests impossible.

To prevent any contamination, mortars, pestles, flasks, large tubes, and glass funnels were cleansed in a concentrated sulphuric acid-potassium bichromate solution overnight, followed by thorough washing in tap and distilled water. Small tubes and other instruments were boiled for 2 hours in a 0.5 per cent solution of sodium carbonate, followed by 4 hours of boiling in distilled water changed four times.

Discussion

Because of the difference in the significance of a positive (+) reading in the precipitation reaction (the "normal precipitin reaction" of KOSTOFF and CHESTER) and the true precipitin reactions, a brief explanation will make the following discussion clearer, particularly so because the precipitin reactions are of long standing in the field of immunology and are therefore better understood.

In the precipitation reaction, as CHESTER (5) has pointed out, an increasing positive (+) reaction between extracts of different species seems to indicate an increasing degree of divergence between such species, while the negative (—) reaction may indicate either very close relationship or great divergence. This is in contrast to the precipitin reaction (involving previous injection of an extract into an animal body and subsequent building up of an immunity to that extract), where an increasing (+) reaction is reported to indicate increasingly closer relationship (MEZ and others), the (—) reaction indicating only divergence.

Since the use of extracts from dried material gave unsatisfactory results in these tests, their reactions, especially negative ones, must be regarded as questionable. It will be necessary to retest the species so used with extracts from green material. Furthermore, the study

includes only half the sections, slightly more than half the subsections of the largest section, and less than one-fifth of the recognized species of the genus. In view of these things, any conclusions that are drawn must be regarded as tentative.

Among the Sibericae, it will be noted that *I. prismatica* reacted more strongly and more frequently than the other two Sibericae studied. DYKES (9, p. 101) has pointed out certain morphological differences that separate *I. clarkei* Baker and *I. prismatica* from the rest of the Sibericae, and has suggested that, in the case of the former at least, these differences are almost great enough to justify the erection of a separate subsection. The more pronounced precipitation reactions of *I. prismatica* seem to support DYKES' suggestion (table I), and still further support is found in the breeding record of *I. prismatica* (4, 13, 17). Crossed with 16 species and varieties with varying success, it has apparently not been crossed successfully with members of the Sibericae, although it has produced seed in crosses with *I. pseudacorus* (Laevigatae) and *I. foliosa* (Hexagonae).

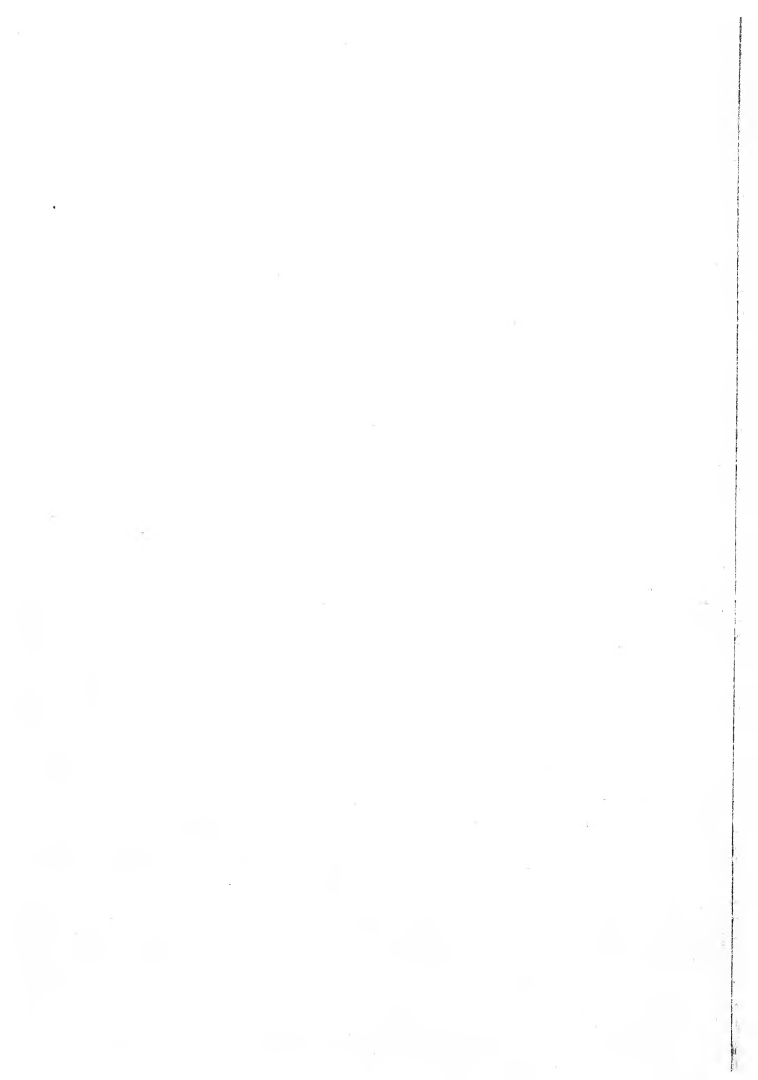
The Laevigatae are outstanding in their reactions. There are positive reactions of varying intensities between them and all other sections and subsections studied, except Vernae (table I). With the Sibericae, however, there is but one positive reaction as compared to ten negative reactions. The preponderance of negative reactions between these two groups might indicate either closeness or divergence of relationship, a decision being facilitated by consideration of the breeding evidence (3, 4, 7, 13, 17). Not including the Sibericae, 125 crosses have been recorded between the Laevigatae and all sections and subsections, of which only 18 were successful. Of 94 crosses between the Laevigatae and the Sibericae, 24 were successful. This latter figure becomes even more significant when it is considered that only 18 out of 44 crosses were reported successful between species of the Laevigatae. Clearly the Laevigatae cross more readily with the Sibericae than with other groups, and even these crosses are more successful when the former are used as pollen parents. In this case, the negative precipitation reactions, supported by a good proportion of successful crosses (considering the percentage of successful crosses between members of the group Laevigatae), suggest a comparatively close relationship between the two groups.

TABLE I
PRECIPITATION REACTIONS IN THE GENUS IRIS
d = DRIED MATERIAL; ± = TRACE; + = 1; ++ = 2; -- = NO REACTION

SECTION	SUBSECTION	IRIS SPECIES															
		orientalis (Snow Queen)	forrestii (d)	prismatica	unguicularis	ochroleuca	halophylla	graminea	kämpferi (Albatross)	pseudacorus	pseudacorus var. bastardi	hexagona foliosa	fulva	fulva X foliosa (D. K. W.)	fulva X foliosa (Fulvala)	chrysophoenicia	violpurpurea
		hyacinthiana	tricuspidata	canadensis	verna	vinicolor	dichotoma	cristata	gradilipes	tectorum	stolonifera var. vago	hoogiana	koehii	pallida X pallida (M. Tinley?)	bucharica		
(I) Apogon†	(1) Sibericae	orientalis (Snow Queen)	forrestii (d)	prismatica	unguicularis	ochroleuca	halophylla	graminea	kämpferi (Albatross)	pseudacorus	pseudacorus var. bastardi	hexagona foliosa	fulva	fulva X foliosa (D. K. W.)	fulva X foliosa (Fulvala)	chrysophoenicia	violpurpurea
	(8) Unguicularae	unguicularis															
	(9) Spuriae	ochroleuca	halophylla	graminea													
	(10) Laevigatae	kämpferi (Albatross)	pseudacorus	pseudacorus var. bastardi	hexagona (d)	foliosa	fulva	fulva X foliosa (D. K. Williamson)	fulva X foliosa (Fulvala) (d)	chrysophoenicia	violpurpurea						
	(11) Hexagone	fulva X foliosa (D. K. Williamson)	fulva X foliosa (Fulvala) (d)	chrysophoenicia	violpurpurea												
	(12) Ensatae	hyacinthiana (d)															
	(14) Tripetalae	tricuspidata (d)	canadensis (d)														
	(15) Vernae	verna (d)															
	(unassigned)	vinicolor															
	(II) Pardanthopsis	dichotoma (d)															
(III) Evansia		cristata	gradilipes (d)	tectorum													
	(V) Regelia	stolonifera var. vago	hoogiana (d)														
(VII) Pogoniris		koehii (d)	pallida X pallida (M. Tinley)														
	(IX) Juno	bucharica (d)															

* Trace did not appear until after 30 minutes.

† Sections and subsections numbered according to ENGLER and PRANTL.



The reactions and breeding results of *I. pseudacorus* (Laevigatae) are more striking than those of any other single species considered here. Of the 11 crosses, reciprocal or otherwise, between *I. pseudacorus* and species, varieties, or hybrids included in this study (table II), eight were unsuccessful. Precipitation reactions between these

TABLE II
CORRELATION BETWEEN BREEDING EVIDENCE AND PRECIPITATION
REACTIONS FOR *I. PSEUDACORUS* AND *I. FULVA*

♀ PARENT	♂ PARENT	RESULT OF CROSS	RECIPROCAL CROSS	REACTION	AUTHORITY FOR CROSSES
pseudacorus	Snow Queen	S	U*	+	13, 17
pseudacorus	prismatica	U	S	—	4, 13
pseudacorus	graminea	U	U	—	4
pseudacorus	hexagona (d)	U	U*	+	17
pseudacorus	foliosa	U	U*	+	4
pseudacorus	fulva	S	U	+	13, 4
pseudacorus	D. K. W.	U	U	+	17, 4
pseudacorus	tricuspis (d)	U*	U*	+	4
pseudacorus	canadensis (d)	U	U	±	4
pseudacorus	vinicolor	S		++	13
pseudacorus	tectorum	U		++	13
fulva	Snow Queen	U*	U*	—	13
fulva	pseudacorus	U	S	+	4, 13
fulva	foliosa	S	S	—	4
fulva	D. K. W.	U	S	—	4
fulva	tricuspis (d)	U	U*	—	4
fulva	canadensis (d)	U	U	—	13, 4
fulva	vinicolor	S	S	—	4, 3
fulva	tectorum	U	U	—	13
prismatica	fulva	U*		—	4
graminea	fulva	U		—	4
pseudacorus var. bastardi	fulva	S		—	4
hexagona (d)	fulva	S		—	13
fulvala (d)	fulva	S		—	4
cristata	fulva	U		—	4

Explanation of symbols: (d) indicates use of extract from dried material. In the columns of breeding results, U indicates failure to produce seed; * either parthenocarp or the production of non-viable seed; and S the formation of viable seed. The reaction symbols are the same as those in table I.

eight different forms and *I. pseudacorus* show one negative, one trace, and six (+) reactions, a rather good correlation with the breeding results. All available records show that *I. pseudacorus*, as a seed parent, has been used successfully in crosses with four other species, unsuccessfully in 22; as a pollen parent it has been used successfully in crosses with nine other species, unsuccessfully in 25. No reciprocal cross is recorded in which it has been successful as

seed and pollen parent. Of these successful crosses with 13 other species, seven were crosses with members of the Sibericae (six if *I. prismatica* is separated from that group) and three were crosses with members of its own subsection, the Laevigatae.

In the crosses between *I. pseudacorus* and *I. orientalis* (Snow Queen), and *I. vinicolor* and *I. fulva* (table II), a new situation arises between the breeding evidence and the precipitation reactions. These species, although crossed with partial success, gave a positive reaction in each case with *I. pseudacorus*. In the case of *I. orientalis*, of six flowers pollinated only one formed a seed capsule, and that one contained few seeds (13, p. 24); the reciprocal cross was a failure. From five flowers pollinated in the cross with *I. vinicolor*, only one capsule was formed; it also held few seeds (13, p. 24). In the case of *I. fulva*, only five capsules were formed out of 42 pollinations, although each capsule contained many seeds (13, p. 24). The positive reaction between such species may well be regarded as an indicator of the relatively low degree of fertility exhibited by such crosses.

No reaction was secured between *I. pseudacorus* and *I. graminea*, and the cross between the species was unsuccessful (4, pp. 35, 37). The negative reaction here indicates divergence of the species.

There is often a rather close parallel between the reactions of *I. vinicolor* and species of the group Hexagonae (table I), especially so with *I. chrysophoenicia* and *I. violipurpurea*, suggesting a relationship between them. SMALL and ALEXANDER (21) have recognized the relationship between *I. vinicolor* and *I. violipurpurea* by placing them, together with several others, in a new group, the Unicristatae, although they associate *I. chrysophoenicia* with another new group, the Coronicristatae. The preliminary evidence from precipitation reactions would scarcely support the segregating of *I. chrysophoenicia*, but it does support the grouping of *I. vinicolor* with *I. violipurpurea*.

The precipitation reactions and breeding results for *I. fulva* (table II) show cases of close relationship to certain species, and of more distant relationship to others. Of the 14 cases cited, seven show successful crosses, six of which are correlated with negative reactions. In the seven unsuccessful crosses, in each instance there is also a negative reaction. If the negative reaction between some species

indicates divergence (and the evidence thus far in this study partly supports the contention of CHESTER in this regard), the failure of crosses between such species as *I. fulva* and *I. tectorum* is not at variance with the precipitation reaction, but on the contrary supports it. This would hold true of other cases, for example, *I. cristata* \times *I. fulva*, *I. tectorum* \times *I. vinicolor*, *I. prismatica* \times *I. tectorum*, etc.

TABLE III

CORRELATION BETWEEN BREEDING EVIDENCE AND PRECIPITATION REACTIONS
FOR *I. FOLIOSA* (D. K. WILLIAMSON), *I. GRAMINEA*, AND *I. PRISMATICA*

♀ PARENT	♂ PARENT	RESULT OF CROSS	RECIPROCAL CROSS	REACTION	AUTHORITY FOR CROSSES
<i>foliosa</i>	Snow Queen	U		±	13
<i>foliosa</i>	<i>pseudacorus</i>	U*	U	+	4
<i>foliosa</i>	D. K. W.	S	S	—	4
<i>foliosa</i>	<i>fulva</i>	S	S	—	4
<i>foliosa</i>	<i>vinicolor</i>	U*	U	—	13, 4
<i>prismatica</i>	<i>foliosa</i>	S		±	4
D. K. W.	<i>prismatica</i>	U		±	17
D. K. W.	<i>pseudacorus</i>	U		+	4, 17
D. K. W.	<i>foliosa</i>	S	U	—	4
D. K. W.	<i>fulva</i>	S	U	—	4
<i>vinicolor</i>	D. K. W.	S		—	4
<i>graminea</i>	<i>pseudacorus</i>	U	U	—	4
<i>graminea</i>	<i>fulva</i>	U		—	4
<i>halophila</i>	<i>graminea</i>	U		±	4
<i>prismatica</i>	Snow Queen	U*		—	13
<i>prismatica</i>	<i>pseudacorus</i>	S	U	—	13, 4
<i>prismatica</i>	<i>foliosa</i>	S		±	4
<i>prismatica</i>	<i>fulva</i>	U*		—	4
<i>prismatica</i>	<i>vinicolor</i>	U*		+	4
<i>prismatica</i>	<i>tectorum</i>	U*		—	13
D. K. W.	<i>prismatica</i>	U		±	17

Symbols are the same as in table II.

Where the morphological differences are great, as in the cases cited, the negative reaction ought almost certainly to be interpreted as indicating divergence.

Table III gives a similar comparison for some of the other irises studied. *I. foliosa* and D. K. Williamson (*I. fulva* \times *I. foliosa*) show a good correlation, while *I. prismatica* and *I. graminea* do not. The last-named species presents certain peculiarities which help to explain such behavior. Table I shows that the reaction between *I.*

graminea and *I. halophila* is the only instance of a reaction between members of the same subsection. Moreover, the chromosome number of *I. graminea* is reported as $n = 17$ (11), differing markedly from other members of the group. In *I. spuria* L., $n = 12$ (11), in *I. ochroleuca*, $n = 20$ (11), and in *I. halophila*, $n = 22$ (11). These four are the only species of the subsection whose chromosome numbers are known, but the reported presence of the uneven number in *I. graminea* can hardly be without significance. No cross utilizing this species is reported as having been successful, although 16 species and varieties were used in the work (4). Even within its own subsection it has not been crossed successfully. The negative breeding results, combined with the generally negative precipitation reactions, are strongly suggestive of a rather solitary position for *I. graminea*.

It was the behavior of embryos in species crosses in *Nicotiana* that suggested to KOSTOFF (15, p. 35) that "immunological phenomena are involved in hybridization and during the ontogeny of hybrid embryos." In regard to *Linum*, LAIBACH (16) has pointed out that the death of embryos in seeds must not be regarded as proof of genetic lethality, since the seed parent may not be qualified to afford normal development to otherwise viable seeds. He states: "Because embryo and mother-plant are mutually adapted in their ontogeny, disturbances in the physiological relations can be produced by genotypical differences that are the reason for the death of the embryo." He reports demonstrating this by removing the hybrid embryos of a cross which does not produce viable seeds, and maturing them successfully on cotton in a sugar solution, growing plants from some of the seeds thus matured. This lends strength to the view that in some instances, certainly, the failure to produce seed is due to the hostile environment in which the embryos are developing.

Similar cases apparently exist in species crosses in *Iris*. Sometimes there is parthenocarpy, and sometimes dead embryos can be found in the seed capsules, for example, in the cross *I. pseudacorus* \times *I. versicolor* (20). The reciprocal cross, *I. versicolor* \times *I. pseudacorus*, will usually produce a few seeds (4, p. 41); and indeed, like the *Laevigatae* in general, *I. pseudacorus* seems rather more successful as a pollen parent than as a seed parent. This failure to produce

viable seed in one direction in a reciprocal cross might be attributed in this case to "endosperm deficiency," as has been done in other similar cases by THOMPSON (22), who suggests that in reciprocal interspecific crosses which differ in the success with which they can be made, the more successful of the pair is that in which the species with the higher chromosome number is used as the seed parent. Since the chromosome number of *I. pseudacorus* is $n=12$ (11), and the chromosome number of *I. versicolor* is reported as ranging from ca. 36 to ca. 56 (11), this explanation seems to apply to the case in question. On the other hand, it seems more reasonable to suppose that this endosperm deficiency is perhaps not the *causa causans* of the death of the embryos, but is itself simply an expression of physiological or immunological incompatibility, as suggested by LAIBACH and KOSTOFF.

The "immunological" interpretation of the precipitation reaction is discouraged by CHESTER (6), who has found strong evidence in certain woody plants against the theory that the reaction is of a protein nature. He reports that nearly 57 per cent of the reactions observed in his work consisted of calcium oxalate precipitations, and states (6, p. 295): "This precipitation of calcium oxalate is so frequently the sole or main phenomenon in the reactions considered immunological in nature as to invalidate immunological interpretations laid on such reactions in which the calcium oxalate factor is not removed." The remaining factors he interprets by assuming the presence or absence of three other pairs of reactive substances. Although this tends to discourage an immunological interpretation in the majority of cases, it is still true that reactive principles of one sort or another are responsible, even though proteins may not be solely or even directly responsible for the reactions. It would be only reasonable to expect that precipitation reactions between species are due quite as much to the presence of other products of metabolism of species as to their proteins. Although it has been determined that the precipitation in *Iris* is not a calcium oxalate reaction, its exact nature is still to be discovered. In any case, the substitution of another factor or factors for the protein factor does not affect the present evidence, namely, that there is a correlation between the precipitation reactions and success or failure in crossing.

The failure of crosses, in some instances at least, is attributable to the inability of the hybrid embryos to develop in a physiologically hostile environment.

The distinctiveness of *I. pseudacorus* is further confirmed by AUGEM (1, 2) in a comparative study of the storage products of the rhizomes of *I. germanica* L., *I. pseudacorus*, and *I. foetidissima* L. He found that the rhizome of *I. germanica* contains saccharose and starch; that of *I. pseudacorus* stores a little saccharose and the levulosan, irisine, but never contains starch; while in the rhizome of *I. foetidissima* are found saccharose, starch, and two levulosans. He concludes that since the three types are so different from one another, physiologically speaking, it may well be because of this fact that crosses between them have so far never produced viable seed. Although in his studies on starch grains REICHERT (18, 19) included no mention of any member of the Apogon section, he found a marked difference between the type of grain represented by Pogoniris and Oncocyclus on the one hand and the three bulbous sections on the other (19, p. 708).

Summary

1. The precipitation reactions between thirty species, varieties, or hybrids are recorded. They represent, in part, six of the twelve sections of the genus *Iris*.

2. As correlated with breeding evidence, positive precipitation reactions between species usually indicate either a low degree of fertility between such species, or complete interspecific sterility.

3. It appears likely that the presence of a trace indicates too divergent a relationship for a positive reaction of any magnitude. As a rule a trace may be correlated with complete interspecific sterility.

4. A negative reaction between species may indicate close relationship, as shown by successful crosses, or it may indicate extreme divergence between species and be correlated with interspecific sterility.

5. Although decidedly limited, the evidence from gross analyses of storage products and that from microscopic examination of starch grains is in line with the precipitation reactions and the breed-

ing results. As a whole, such evidence supports the present taxonomic grouping, except in the following:

a) *I. prismatica* is divergent from the subsection *Sibericae* of the *Apogon* section, as evidenced by gross morphology, breeding results, and precipitation reactions.

b) *I. pseudacorus*, considered in the light of all the available evidence, seems even more distinct from other irises in the *Apogon* section than its present taxonomic position would indicate.

c) *I. vinicolor* in its precipitation reactions is so similar to the *Hexagonae* that its inclusion in the group, or with certain members usually assigned to the group, seems warranted.

d) *I. graminea*, in the light of all available evidence, should occupy a rather solitary position.

e) The subsection *Laevigatae* shares the distinctiveness of its individual member, *I. pseudacorus*. There are positive reactions of varying intensities between the *Laevigatae* and all other sections and subsections studied, except *Vernae*. With the *Sibericae*, however, there is but one positive reaction as contrasted with ten negative reactions, a situation which shows a marked correlation with breeding results, and suggests a comparatively close relationship between the two groups.

6. The precipitation reaction is discussed in the light of chromosome numbers, breeding evidence, and immunological and other phenomena as involved in hybridization and during the ontogeny of hybrid embryos.

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GERMINATION BEHAVIOR OF MAGNOLIA GRANDIFLORA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 442

CLYTEE R. EVANS

Introduction

Magnolia grandiflora (L.) belongs to a very old genus now limited to southeastern North America, southern Mexico, and southern and eastern Asia. It is found in the coastal plain of the southeastern part of the United States. Within this area it grows in rich moist soil on the borders of river swamps and ponds, particularly on hammocks in Florida and fertile rolling slopes along the Mississippi River.

In the literature on seed germination there is very little reference to the behavior of the various species of *Magnolia*. MILLAIS (30) mentions the ready germination of *Magnolia macrophylla* if the seeds are sown immediately after being gathered. He refers to the seeds of certain Chinese species as short-lived, being capable of germination for only a very short time after they are shed. He suggests for seeds of *M. grandiflora* the treatment outlined by BAILEY (2). This method calls for storage of the freshly gathered seeds in dry sand until February, then in moist sand for a week or ten days to loosen the outer fleshy coat. After these coats are removed by washing in water, the seeds are sown in a cold frame. TOUMEY (44) found that the seeds of *M. acuminata* give a germination of only about 3 per cent at maturity, although 68 per cent seem sound. He believes the hard endosperm of these seeds requires several months for the absorption of sufficient water for germination. Observations of a botanist in Florida are to the effect that seeds of *M. grandiflora* germinate in moist situations in the shade of the trees in great numbers in the spring following their shedding in the fall. The writer has observed that seeds of this species when shed from the tree germinate very rarely although they are viable. Probably the limits of the natural range may be related to the germination behavior of the seed.

Nurserymen in the region where *Magnolia grandiflora* grows advise macerating the seeds, stratifying them in moist sand at low temperatures until spring, and then planting. They say that if the seeds dry out they will lie over a year, if they come up at all.

The chemical composition of the seed and the changes taking place in the endosperm and embryo during germination have had almost no attention. WEHMER (47) mentions a fat and an ethereal oil, magnoliol, as having been determined in the seed. Alkaloids, glucosides, resin, and saponin have been claimed on evidence not altogether convincing. Studies such as those by TOOLE (43) on maize seeds and ECKERSON (18) on wheat seedlings interested the writer to attempt to study the chemical changes in the seeds of *M. grandiflora* during normal germination under a given set of conditions. It was believed also that these studies might point the way toward a method of overcoming their dormancy.

Many attempts were made to hasten the after-ripening or to overcome dormancy. A wide variety of conditions was used, as will be indicated in the section dealing with experimental work, but without marked success. A summary of the extensive literature which guided the work is not attempted here.

During the studies of early germination behavior, the writer became interested in the oxidation-reduction mechanisms of the embryos and the food reserves. This interest centered in the activity of oxidase and catalase and in the glutathione mechanism. This compound, which HOPKINS (24, 25) discovered and considered a dipeptide of cysteine and glutamic acid, has been reinterpreted by him as a tripeptide including glycine, as well as the two amino acid components first discovered. Since 1921 it has been considered by many workers in animal physiology as having an important rôle in respiration, being present in greatest quantities in tissues having a high metabolic rate. It has been found to be widely distributed in plant tissues also. Up to the present time only slight attention has been given it in connection with studies on seed germination. FIRKERT and COMHAIRE (20) have demonstrated its presence not only in active meristematic regions but also in reserves. Of particular interest is the fact that they studied the increase in glutathione in seeds of the pea during absorption of water. This formation of glutathione

has been attributed by VIVARIO and LE CLOUX (46) to the hydrolytic decomposition of polypeptide complexes. These studies, together with such work as that of KOZLOWSKI (28) on the distribution of non-protein cysteine in meristems and other plant tissues, and that of CAMP (7) on the shift in metabolic activity in flower primordia, determined quantitatively on the basis of the nitroprusside color test for glutathione, made it seem particularly desirable to study the glutathione content of *Magnolia grandiflora* seeds during germination. Moreover, interest in compounds containing -SH groups has been stimulated by work done at the Lankenau Hospital in Philadelphia on the healing properties of such compounds as thioglucose, cysteine, and thioglycollic acid. Certain of these have been found to stimulate growth, actually causing an increase in cell division in root tips. It seemed that these compounds might have some significance in the problem of dormancy, such as is exhibited by *Magnolia* seeds.

This paper presents the results of a study of the behavior of seeds of *M. grandiflora* during the preparation for germination and the early stages of the process, with particular reference to the overcoming of dormancy, to the chemical changes taking place during after-ripening, and to the presence and development of oxidizing mechanisms, especially glutathione.

Materials and method

The seeds used in these experiments were of four lots. Lot 1 was 1928 seed received in the pulp nine months after being gathered. Lots 2, 3, and 4 were all 1929 seed. Lot 3 was gathered by the writer in Mississippi in October, 1929; lot 2 was received approximately two months after being harvested; lot 4, seven months afterward. Some seeds of each lot were stored dry at room temperature in glass jars; some of lots 2, 1, and 4, moist at 10°C.; and some of lot 1 were stored dry at 10°. Lot 4 was infected with a green mold when received. Preliminary to storage in damp sterile sand at 10°, the seeds were macerated, after soaking in water 24 hours, and disinfected in CaOCl₂ solution.

STRUCTURE.—Observations were made on the gross structure of the seed and seedling and on the course of development of the latter. These were followed by microscopic and microchemical studies of

freehand sections of which camera lucida drawings were made in many instances.

GERMINATION STUDIES.—All seeds in dry storage were macerated and soaked for two hours in CaOCl_2 solution prepared as suggested by WILSON (48). Tests were run using the seed with the lignified coat on, and others with it removed on one side. Embryos free from the endosperm were set to germinate also. The temperatures used were 10° , 20° , 23° , 29° , and an alternation of 10° and 23°C . The many chemicals used were those suggested by the results of other investigations on problems of dormancy or stimulation.

CHEMICAL CHANGES.—Seeds germinated in the jars of moist sand stored at 10° were studied intensively in an attempt to determine particularly the changes taking place between the splitting of the coat and the emergence of the hypocotyl. In many cases these studies were extended to include the dry seeds of the same lot and seedlings older than the two stages just mentioned. The microchemical tests used were largely the ones for reducing sugars, starch, amino acids, reserve proteins, soluble proteins, fats, and a few others given by ECKERSON (16) or TUNMANN (45). Free acids were determined by titration with NaOH , using phenolphthalein as the indicator. Neutral red was used to detect localization of changes in reaction in the endosperm and embryo.

OXIDIZING ENZYMES AND GLUTATHIONE.—For the determination of the activity of oxidase, peroxidase, and catalase, qualitative tests suggested by ECKERSON (16) were used. These were checked further by quantitative determinations of oxidase according to BUNZELL (6) and catalase by the APPLEMAN method (1). In testing for glutathione, the nitroprusside method of HOPKINS as used by FINK (19) was adopted. Sections of the seeds were heated in dilute acetic acid to the boiling point and then placed in shallow dishes in enough saturated ammonium sulphate to cover them well. Immediately a few drops of a 5 per cent solution of sodium nitroprusside $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ were added and the material allowed to stand for at least five minutes. Then 1 cc. of ammonium hydroxide, full strength, was added. The appearance of pink to permanganate color was considered to indicate the presence of glutathione. The

depth of the color and the length of time it remained indicated the amount present. The glutathione test was run on seeds of *Ricinus* also.

Results

STRUCTURE

Diagrams of the structure of the seeds of *Magnolia* have been published by BRANDZA (3) and SARGENT (41). BRANDZA interprets the fleshy outer coat as the testa; the inner lignified one as the tegmen; and the membranous innermost one as the remnant of the nucellus. SARGENT calls the membranous coat the tegmen, and considers the lignified one and the fleshy one as parts of the testa. BRANDZA's opinion is accepted here. The fleshy outer coat is made up of three regions: a three-layered epidermis, a wide region of at least six rows of large fleshy cells containing much oil, and within this a layer of small cells forming a sort of inner epidermis. This fleshy coat contains 57 per cent oil and a considerable quantity of reducing sugars. It is traversed by the vascular bundle which leads through the raphe to the chalaza, a region where the coats are undiverged. The lignified coat is made up of radial rows of heavy-walled cells. The nucellus is a membranous layer, brown in color and easily plasmolyzed by the many reagents used in the microchemical tests described elsewhere in this paper. The chalaza and the micropyle lie in a line, the hilum a little to one side of the micropyle.

The endosperm is massive, with a deep groove on one side into which fits a projection of the lignified coat. The cells are large thin-walled ones containing protoplasm packed with both protein reserve bodies (with globoid-like inclusions) and numerous small globules of oil, but no starch grains. The oil content tests 51 per cent. This region gives a striking phytosterol test.

The embryo is extremely small, being 1 mm. in length and 0.4 mm. in diameter. In the freshly gathered seed it shows the same degree of differentiation as it does when the coat splits preliminary to germination, although at this stage it has almost doubled in dimensions. It consists of a hypocotyl and two leaflike cotyledons between which lies a mass of undifferentiated cells, the plumule.

In the germinating seed the shift in the rate of growth is about as

follows. The cotyledons invade the endosperm and extend approximately throughout the length of it before the hypocotyl emerges. In this process one cotyledon usually increases in size more rapidly than the other. Cell contents and cell walls disappear as these cotyledons grow. Soon the hypocotyl takes the lead. First the basal portion pushes out through the endosperm and nucellus; then the upper portion grows rapidly and arches upward, pulling the cotyledons from the remnant of the endosperm. Regularly by this time four secondary roots have appeared in the transition region between root and stem. The cotyledons remain on the plant for four months or more, by which time the plant has acquired seven or eight other leaves.

An interesting change in correlation occurred in 40 per cent of the seedlings developing from seeds previously treated with NaCNS. The upper portion of the hypocotyl pulled the cotyledons out of the endosperm before the emergence of the lower end of the hypocotyl.

GERMINATION TESTS: POSITIVE RESULTS

Tables I and II indicate the type of results with 1929 seed. Each shows the general increase in percentage germination given by these seeds when provided the temperature alternation of 10° and 23°C . The various compounds used in the attempt to increase the percentages of germination or decrease the time required had no marked effect. With one exception the seeds germinated in as high percentage when provided distilled water. This result, the 50 per cent germination secured in one culture with N/5000 zinc sulphate, was probably a chance one as it could not be repeated. No germination was secured in the presence of many compounds tried. A list of these is given.

The time required for germination of seeds with the lignified coats removed from one side at the highest temperature tried, 29° , was 12 days as compared with 30 days required at the alternation and at 20°C . The total percentage was greatest at the alternation.

The time required for germination at 20° or 23° is shortened by storage at 10° in moist sand for 6-10 weeks, whereas a continuance of this storage for 18 weeks seemed less effective, as then the seeds required a longer time to germinate at the same temperature. The increase in time required may be due to the lowering of vitality by

the CaOCl_2 used in disinfecting the seeds. Some of it remains in the chalazal region.

Seeds with the lignified coats on when set to germinate at 23° required nine weeks; those with this coat off on one side required from three to four weeks at the same temperature.

TABLE I

PERCENTAGE GERMINATION GIVEN BY SEEDS OF LOT 2 (SEEDS RECEIVED
IN PULP; PREVIOUS STORAGE CONDITIONS NOT KNOWN, BUT
PROBABLY DRY AT AIR TEMPERATURE FOR TWO MONTHS)

STORAGE CONDITIONS AFTER RECEIPT	TEMPERATURE AT WHICH SET TO GERMINATE	MEDIUM	LIGNIFIED COAT ON OR OFF ON ONE SIDE	PERCENTAGE GERMINA- TION
10° , moist sand for 30 days..	Alternation of 10° and 23°	$\text{N}/100\text{KNO}_3$	Off	15
Dry at room temperature for 30 days.....	Alternation of 10° and 23°	H_2O	"	30
Dry at room temperature for 30 days.....	Alternation of 10° and 23°	H_2O	"	25
Dry at room temperature for 30 days.....	Alternation of 10° and 23°	H_2O	"	35
Dry at room temperature for 30 days.....	23°	$\text{N}/2500\text{MnSO}_4$	"	10
Dry at room temperature for 30 days.....	"	H_2O	"	5
10° , moist sand for 18 days	"	$\text{N}/5000\text{ZnSO}_4$	"	10
" " " " " "	"	H_2O	"	15
" " " " 24 " "	"	H_2O_2	"	5
Dry for time.....	"	1% NaCNS	"	15
10° , moist sand for 24 days..	"	$\text{N}/5000\text{ZnSO}_4$	"	10
" " " " " "	"	"	"	15
Dry.....	"	"	"	5
" " " " " "	"	H_2O	"	5
10° , moist sand for 38 days..	"	$\text{N}/5000\text{ZnSO}_4$	"	10
" " " " 43 " "	"	H_2O	"	5
" " " " 18 " "	20°	H_2O	On	10
" " " " 38 " "	"	H_2O	"	25
" " " " 18 " "	"	$\text{N}/5000\text{ZnSO}_4$	Off	15
" " " " 40 " "	"	$\text{N}/500\text{ZnSO}_4$	"	10

Seeds stored in moist sand at about 10° started germinating in 16 weeks and continued this for eight more weeks, when the number began decreasing. Those planted in garden soil in the greenhouse at

a temperature of about 25°C. within a week after being gathered began germinating in 14 weeks.

TABLE II
PERCENTAGE GERMINATION GIVEN BY SEEDS OF LOT 3

STORAGE CONDITIONS	TEMPERATURE AT WHICH SET TO GERMINATE	MEDIUM	LIGNIFIED COAT ON OR OFF ON ONE SIDE	PERCENTAGE GERMINA- TION
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H ₂ O	Off	30
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H ₂ O	"	40
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	N/5000 ZnSO ₄	"	20
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	NaCNS	"	25
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H ₂ O	On	35
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	Alternation of 10° and 23°	H ₂ O	Off	40
Dry at room temperature for 18 days then in moist petri dishes at 10° for 80 days..	23°	H ₂ O	"	30
Dry at room temperature 100 days.....	Alternation of 10° and 23°	H ₂ O	"	35
Dry for 18 days.....	20°	H ₂ O	"	10
" " " "	"	H ₂ O	"	20
" " " "	"	N/800 butyric acid	"	15
" " " "	"	N/1000 acetic acid	"	10
" " 32 "	"	N/500 ZnSO ₄	"	10
" " 39 "	"	H ₂ O	"	10
" " 45 "	"	H ₂ O	"	10
" " " "	"	N/5000 ZnSO ₄	"	50
" " " "	"	N/100 NaNO ₃	"	5
" " " "	"	1% NaCNS	"	10
" " " "	"	2% ethylene chlorhydrin	"	5

GERMINATION TESTS: NEGATIVE RESULTS

Seeds stratified in moist sand were subjected for 48 hours to a temperature below freezing which caused grains of sand to cling lightly together. All seeds whether their coats were already split or still intact were killed.

Lots 1 and 4 did not germinate at all. Both were apparently sound. The behavior of each differed when stratified and again when set to germinate at 23° from that of lots 2 and 3. Lot 1, the 1928 seed, absorbed less water, 31.2 per cent as compared with 39 per cent absorbed by lot 3. Upon the seeds of lot 4, seeds received from a dealer seven months after they were gathered, blister-like elevations formed, raising the nucellus. These burst after a day or two, liberating a fluid giving the Flückiger test for reducing sugars. Disintegration of the mealy endosperm followed quickly.

Seeds of lot 1 were tested on cellucotton moistened with N/10 HCl, N/10 $\text{H}_2\text{C}_2\text{O}_4$, N/500 ZnSO_4 , N/100 butyric acid, N/1000 acetic acid, N/3400 HCl, and 10 per cent dioxygen. Seeds of lots 2 and 3, with lignified coats on or off, gave negative results on N/3400 and N/6800 HCl, N/1600 butyric acid, N/2000 acetic acid, N/5000 MnSO_4 , 5 per cent KCl, 1.5 per cent acid monopotassium phosphate, 5 per cent glucose, Knop's nutrient solution, sterilized extract of the outer coat, and castor bean lipase plus N/1000 acetic acid. These same seeds failed to respond to soaking for 30 minutes in these reagents: 1 per cent thiourea, 2 per cent KCNS, 2 per cent chloral hydrate, and chloroform in the dilution of one drop in 100 cc. of H_2O .

Embryos removed from the endosperm of dry seeds did not grow on glucose, Knop's solution, sterilized extract of the outer coat, 2 per cent dioxygen, or distilled water.

CHEMICAL CHANGES

REDUCING SUGARS.—In the dry seed there is a trace of glucose in the endosperm directly surrounding the embryo, in the distal half of each cotyledon, and throughout the epidermis of the cotyledons. As the coats split, the first noticeable increase is in the endosperm around the whole embryo. As the seed reaches the stage when the cotyledons are actively invading the endosperm, reducing sugars become more abundant in the periphery of the embryo and occur also

in the plumule and in the cells near the vascular tissues of the hypocotyl. At this stage there is a trace of glucose throughout the endosperm and enough present in some cells near the embryo to give ready formation of osazones. At the time of withdrawal of the cotyledons from the remnant of endosperm, reducing sugars are abundant in the phloem and endodermis of the root and stem, and are present throughout the seedling. In this remnant of the endosperm there is a considerable quantity of reducing sugar, together with much oil and a little protein. Reducing sugars are therefore present in both endosperm and embryo of the dry seed. As the seed prepares for germination these substances increase in both regions, notably in the endosperm near the embryo, in the epidermis of the embryo, in the cotyledons, and in meristem in general. No mashing or packing of cell walls of depleted cells takes place; the walls are digested.

SUCROSE.—Sucrose was found in a few instances with the glucose, but in very small quantities. In all tests greater quantities were found when the hydrolyzing agent was citric acid than when it was invertase. This may indicate the presence of glucosides which were hydrolyzed by the acid but were not affected by the invertase.

STARCH.—Starch was not found in either the endosperm or in the embryo of a dry seed. It was not present in one stratified in damp sand at a temperature of about 10° for 14 weeks or in one with coats barely split. It appears in the mesophyll of the cotyledons as they begin their growth, however, and increases rapidly in amount. Soon the cotyledons become packed throughout, with the exception of an area at the tip and the epidermal cells where none ever appeared. At this stage in some seeds both the endosperm cells lying between the cotyledons of the embryo and others touching it contained starch grains. Before the hypocotyl has pushed out, it also shows considerable starch except in cells of the growing point. It occurs abundantly and in large aggregates of grains in the pith, in pith rays, and in the region of the pericycle and endodermis. Scattered small grains of starch were present everywhere throughout the cortex. By the time the hypocotyl has extended the length of the seed, starch has increased throughout the embryo and become noticeable in some epidermal cells. Root hairs exhibit groups but all growing points are free of them. Starch remains abundant in the embryo until the up-

per part of the hypocotyl begins the rapid growth and arching which draws the cotyledons out from the small remnant of endosperm; then it begins decreasing. At the time of withdrawal of the cotyledons it is present in small quantities in the pericycle, endodermis, mesophyll of cotyledons along veins, and all tissues just below the plumule.

OIL.—There is a great amount of oil in the seed of *Magnolia grandiflora*, 51 per cent on the basis of vacuum-oven dry weight of the seed with coat removed. It is present in both endosperm and embryo in all stages studied, even in those cells of the endosperm touching the embryo, which in the dry seed were found almost free of protein reserves, presumably depleted by the embryo in its development. In the dry seed the oil tends to collect in large globules when mashed out of the cells. In the imbibed seed with coats barely split the oil exhibits the same characteristics and distribution as in the dry one. With the decided growth of the cotyledons, however, followed soon by the pushing out of the hypocotyl, changes in amount and size of the globules become apparent. There is a decrease of oil in the embryo, while in the endosperm it disappears slowly from the cells touching the embryo and a few rows beyond these. At this time there exists a gradation from cells next to the embryo in which there is only a small globule or none at all, through those with large globules, outward to cells with the whole protoplast crowded with them. In these cells which are almost free of oil the protein is completely gone while the cell wall is yet intact. It also finally disappears. Oil globules mashed out of such cells in which oil seems disappearing aggregate in much smaller globules than those from the endosperm of dry seeds, but are not of the size PACK (37) describes and considers a translocation form. RHINE (39), however, denies a translocation form of fat in the fatty seeds investigated. Oil is present in all cells but is not so abundant as in younger embryos, particularly in those of dry seeds. The decrease in oil seems correlated with the increase in starch and reducing sugars already noted.

RESERVE PROTEINS.—The endosperm cells in the dry grain, with the exception of an area about six cells wide around the embryo, are packed with protein grains, each containing a body which is colorless when treated with picronigrosin. In these cells the protein reserves occur in a decreased amount or are even almost absent in those cells

touching the embryo. Table III summarizes the tests applied to these grains of protein and the amino acid content of the proteins as indicated by them.

The bromine test for free tryptophane gave negative results.

The fewer granules in cells giving the test for soluble proteins are larger than in cells packed with them, and are also much less dense in appearance. In the endosperm of a seed with the cotyledons actively invading it, there is an increasing gradation outward in number of reserve protein grains parallel to that described for oil globules. This same area shows an inverse gradation with reference to soluble proteins.

TABLE III

TEST	AMINO ACID RESPONSIBLE FOR POSITIVE TEST
Xanthoproteic.....	{ Tryptophane Tyrosin Phenylalanine
Millon's.....	Tyrosin
Bromine.....	Histidine
Formaldehyde and H_2SO_4	Tryptophane

SOLUBLE PROTEINS.—By means of the Biuret test soluble proteins were demonstrated. They occurred in small quantities in all seeds tested, whether dry, imbibed, or germinating, and were found most conspicuous in amount in the cells near the embryo in which reserve proteins were scarce. As the seed coat splits, the embryo first shows the test throughout with a deeper color in the root tip, plumule, and epidermis of the whole embryo, and later in the phloem of the hypocotyl and throughout the cotyledons. At the time of emergence of the hypocotyl a striking test is exhibited by the whole of the remainder of the endosperm. The fact that the cotyledons give only a slight test is puzzling. As asparagin gives the Biuret test, these results can probably be interpreted from that standpoint also.

AMINO ACIDS AND ASPARAGIN.—No amino acids could be crystallized out of the dry seed. In tests on the germinating seed, leucine and asparagin were determined, together with an unidentified amino acid which formed broad needle-like crystals. Apparently there was an increase in these with increase in growth of the embryo. No attempt at localization of these acids was made. Quantitative studies could not be carried out because of scarcity of germinating seeds.

REACTION.—The endosperm of a seed with its coats split gave an alkaline reaction with neutral red; the embryo, a very slight acid reaction in the periphery. With further growth this acidity increases in the epidermis of the embryo and becomes noticeable in the end of the hypocotyl and in its vascular regions, as well as in a region of the endosperm surrounding the embryo. The plumule and the pith cells in the upper region of the hypocotyl are still alkaline in an embryo with its hypocotyl 1 cm. in length. -

Tests for free acid in seeds ground with sand and transferred to boiled distilled water were made by titration with N/NaOH, using phenolphthalein as indicator. Table IV presents the data. Three seeds were used in each determination.

TABLE IV
CONDITION OF SEED CC. OF N/NaOH NECESSARY TO NEUTRALIZE
FREE ACIDS PRESENT

Coats barely split.....	0.096
Coats widely split.....	0.289
Hypocotyls 1 cm. long.....	0.395

OTHER TESTS: NITRATES.—No evidence of nitrates could be secured in the tests on various stages studied. Seedlings with hypocotyls more than 1 cm. long were not available at the time these were run.

IRON.—Iron was demonstrated in both endosperm and embryo of dry seeds, and showed no change in distribution in one with coats split. In a seedling having the hypocotyl 8 cm. long a striking localization appeared in the root tips and in the cambium of the hypocotyl. Iron was present in the cotyledons but not in such quantities along the veins as could be expected from MOLISCH's results (32).

SILICON.—Tests with phenol crystals failed to show any silicon. This test was carried out in attempting to determine the composition of the heavy walls of the cortical parenchyma and pith of the seedlings.

PHYTOSTEROL.—The protoplasmic contents of the endosperm cells of all seeds gave a decided test for phytosterol with ether and H_2SO_4 .

ENZYMES AND GLUTATHIONE

Peroxidase and catalase occurred in small amounts in dry seeds and increased during germination. Oxidase, on the other hand, al-

though not indicated by the microchemical tests on either dry seeds or on those with the coats barely split, was most evident in later stages, appearing in the epidermis of the cotyledons and in the endosperm contiguous to them. Glutathione, however, is present throughout the endosperm of a seed with the coat split. Table V shows the distribution and quantity of glutathione in seeds and seedlings of

TABLE V
DISTRIBUTION OF GLUTATHIONE IN SEEDS AND SEEDLINGS
OF *MAGNOLIA GRANDIFLORA*

MATERIAL	PERCENTAGE WATER CONTENT	COLOR GIVEN BY			
		HYPOCOTYL	COTYLEDONS	PLUMULE	ENDOSPERM
Dry seed.....	6	—	—	—	—
Soaked seed.....	37	—	—	—	+
Seed with coats barely split.....	49	+	—	—	+
Seed with coats well split but hypocotyl not protruding.....	61	+	+	—	++
Hypocotyls 1 cm. long...	Not determined	++	—	—	+++
Hypocotyls 2 cm. long...	Not determined	++	Veins ++	+	++++
Hypocotyls 5 cm. long...	Not determined	+	+	+	++

Magnolia grandiflora, as indicated by the depth of color of the nitroprusside test.

Glutathione appears first in the lower end of the hypocotyl, then in the phloem of the vascular region. Its first appearance in the cotyledons is in the veins. When it is somewhat abundant in the hypocotyl in general it is present in greatest amounts just above the root cap and in the phloem regions. Parallel tests run on *Ricinus* seeds showed the same general distribution. The amount indicated in the meristem of the root and in the phloem, however, was far greater than in the reserves.

Table VI gives results of the quantitative determination of oxidase activity. Other tests should be run on the same types of materi-

al and on dry seeds without pyrocatechin in the apparatus to measure the autoxidative activity of substances present in them.

Table VII shows the catalase findings reduced to standard conditions. A range is given in each case showing the lowest amount

TABLE VI
OXIDASE DETERMINATIONS

MATERIAL	CC. OF O ₂ REMOVED IN 10 HOURS MEASURED IN CC. HG
Six seeds with coats barely split.....	1.6
Six seeds with cotyledons invading endosperm.	7.4
Reagents alone.....	0.2

TABLE VII
CATALASE ACTIVITY

MATERIAL	CC. OF O ₂ LIBERATED FROM 10 CC. OF DIOXYGEN BY 3 SEEDS IN 10 MINUTES	
	LEAST AMOUNT DETERMINED	GREATEST AMOUNT DETERMINED
Dry grains soaked 24 hours.....	11.80	16.70
Dry grains which would not swell appreci- ably.....	3.90	9.40
Stored at 12° for 5 months but not germinat- ing.....	7.79	10.85
Endosperm showing, seed split.....	16.70	24.67
Endosperm showing considerably.....	19.20	27.10
Hypocotyls barely emerging.....	48.36	54.20
Hypocotyls less than 1 cm. long.....	66.12
Hypocotyls longer, 2-6 cm.....	78.76
Endosperms of dry soaked grains.....	11.35	14.80
Embryos of dry soaked grains.....	2.06	3.15
Endosperms of seed with coat barely split. . .	13.80	18.75
Embryos of seed with coat barely split.....	2.96	5.92

found and the highest. No attempts were made to determine the localization of the catalase other than to discover whether it is present in both endosperm and embryo.

Peroxidase seemed localized in the embryo end of the seed in both the dry ones and those with the coats barely split. It, also, increases during early germination stages.

Discussion

GERMINATION TESTS

The germination tests revealed very little of interest. The fact that seeds and young seedlings cannot endure a light freeze for 48 hours accounts for the narrow range of the tree in nature.

The cause of the delay in germination lies partly, but not wholly, in the lignified seed coat. Removal of this coat allows a low percentage of germination in three or four weeks' time, in seeds which require from two to three times as long with the coat on, or which do not germinate at all. Failure to germinate in such cases is due usually to death of the seed caused by invasion by fungi. Other causes for delay have not been determined. Whatever they may be, they are removed in many seeds by subjecting them to alternating temperatures. HARRINGTON (23) discusses the various theories put forward by others, and advances a theory of his own to explain the mechanism of breaking dormancy by alternating temperatures. He thinks that possibly at low temperature there may be an accumulation and actual metabolism of oxygen in a form which becomes available for inception of growth at higher temperatures. MORINAGA (34, 33) believes we have yet to learn the mechanism or mechanisms of the action of alternating temperatures and their substituting factors. His evidence seems to indicate that the effect is on the embryo. The effect of alternating temperatures on the germination of *Magnolia grandiflora* may be on the nucellus, on the endosperm, on the embryo, or on more than one of these structures. In this connection the 50 per cent reduction of gaseous exchange in *Ambrosia* seeds by the nucellus at 30°C. found by DAVIS (11) is of interest. He believes dormancy in seeds may be caused by the coats restricting the oxygen supply just below the amount requisite for germination at the temperature used. Moreover, he (12) induced dormancy in seeds of *Xanthium* by this method. In oily seeds probably oxygen is important in the formation from the fats of more or less unstable compounds which are usable by the embryo in germination.

None of the compounds reported by others as forcing agents had any decided value. It is not known whether or not they penetrated the nucellus. Interesting among these are the following: nitrates,

sulphates, and acetic acid which ROSE (40) found to force *Sambucus* seed; dextrose and extract of the fleshy outer coat which IVES (27) found to force holly seeds; weak acids which ECKERSON (17) found forced *Crataegus* seeds; KNO_3 and MnSO_4 for which POPOFF (38) and others claim much; ethylene chlorhydrin, thiourea, and NaCNS found of value by DENNY (13, 14) in breaking the dormancy of potatoes (possibly through inducing the production of oxidizing enzymes in greater quantity or of greater activity); increased oxygen supply secured through supplying H_2O_2 to the seed bed, as found valuable by SHULL (42); and light as found stimulating by GARDNER (21).

Why the seeds failed to germinate at all in the presence of some of the reagents is puzzling. In some cases their death was caused by rapid and vigorous growth of mold on the seed in presence of the reagents.

These studies on germination serve to point out the necessity of determining such matters as the permeability of the nucellus and of the endosperm cells to oxygen, CO_2 , and various chemicals; the factors influencing the water-absorbing power of the endosperm and the embryo; the effect of other temperature alternations on the germination of freshly gathered seeds and on those variously stored, etc.

CHEMICAL CHANGES AND OXIDATION SYSTEMS

The chemical changes studied in seeds of *Magnolia grandiflora* are especially those occurring between the time of splitting of the seed coat and protrusion of the hypocotyl. The interval between setting to germinate and splitting of the coat was 16 weeks at 10° , and six weeks with alternation of temperature; that between splitting the coat and protrusion of the hypocotyl ranges from six days at 23° to 21 days at 10°C . During this latter interval the trend of both transformations and translocations taking place during the preparation for germination is the same as workers in this field have demonstrated for other seeds. The time required may differ greatly. ECKERSON (17) expressed this idea clearly in stating that *Crataegus* goes through the same changes during the 90 days of after-ripening as are telescoped into the first eight days of germination of *Ricinus* as described by DELEANO. IVES (27), also, says that the changes

during the long time needed for growth of the immature embryo of *Ilex opaca* are identical with those enumerated by PACK (35) as accompanying after-ripening in *Juniperus*. DAVIS (10) reported results similar to PACK'S (35, 36). She thinks of after-ripening changes as the accumulation of readily usable materials such as sucrose, starch, amino acids, and soluble proteins, whose utilization by the seedling begins with germination and is accompanied by further breakdown of other less easily hydrolyzable reserves. In *M. grandiflora* the accumulation of the starch and soluble proteins in the embryo apparently follows the hydrolysis of reserve proteins and oil in the endosperm cells adjoining the embryo. Although the embryo itself possesses very meager reserves of its own upon which to draw, it seems slow in hydrolyzing its oil reserves. Soluble proteins and reducing sugars in slight quantities are present in it before there is evidence of active changes in the endosperm. It is to be noted, too, that the disappearance of reserve proteins and oil invariably begins next to the embryo, indicating possibly that the embryo somehow initiates these transformations. Questions to be answered here are, how does the embryo effect this, and what stimulates it to activity? These will be discussed later.

Of particular significance and interest are the observations on the disappearance of both protein and oil reserves. The protein bodies swell, become less dense, and disappear entirely. DAVIS (10) and LAKON (29) report similar changes in size of protein granules in storage cells of other seeds. In the cells depleted of protein granules, soluble proteins are strikingly indicated by the pinkish purple color obtained in the cells by the application of the test. Physiologically the seed of *Magnolia grandiflora* resembles that of *Fraxinus excelsior*, as described by LAKON (29), in that in each one a well differentiated embryo grows within the endosperm simultaneously with depletion of proteins stored there. LAKON calls this growth, this preparation for germination, "Vorkeimung," and states that it will occur in six months in moist sand. At the end of this time the seed germinates; that is, the hypocotyl emerges through the inclosing structures. Removing the coats did not overcome the dormancy. Starch appeared in the embryo within ten days after it was put in the sand, but was never present in the endosperm. The reserve proteins became muc-

laminous before disappearing. LAKON thinks they are glyco-proteins which give rise to carbohydrates in their breakdown, as well as to the commonly accepted protein decomposition products.

Clearly in *M. grandiflora* the soluble proteins are derived from the protein reserves. These shorter-chain protein compounds, on being hydrolyzed further to amino acids, pass into the embryo where resynthesis quickly occurs. BROWN and MORRIS (4) consider that the soluble proteins of barley seeds pass very early into the embryo as it swells, and serve there for the development of enzymes. This may be the situation in the seed of *M. grandiflora*, for certainly enzymatic activity of the epidermis of the embryo seems in some way awakened or greatly increased. Of course these proteolytic products of the reserve proteins serve as material for building protoplasm.

Fats also disappear from the endosperm cells adjoining the embryo. Great stores of starch appear in the mesophyll of the cotyledons as this happens. Cell walls also are being broken down. It seems strange that greater quantities of reducing sugar could not be demonstrated in the endosperm and in the cotyledons at this time. The fact that starch can be found in the endosperm cells now and then during disappearance of fat is interesting in this connection. *M. grandiflora* must have very active diastases. If the carbohydrate-like fragments of the fats, whatever they may be, are not used quickly as respiratory or building materials, they seem almost immediately to be condensed and stored as starch. Starch appears later in other parts of the embryo and is always present near growing points but not in them. PACK (36) thinks that in *Juniperus* both fats and proteins contribute to the carbohydrates found.

The acidity accompanying hydrolysis of fats and proteins in *M. grandiflora* can hardly be related in a causal way to the digestion of both, for, in general, lipase has its optimum pH range from 4.0 to 8.6; and plant trypsins from 7.0 to 9.5. Yet it must be remembered that pepsins have an acid optimum range, and that it is short-chain proteins that are abundant and not amino acids. The acidity is probably due to the fatty acids released. Increases in acidity during the germination of oily seeds have been reported time and again, notably by GREEN (22) who discovered lipase, by MILLER (31) in

his work on sunflower seeds, and by IVANOW (26) and ECKERSON (17).

No accumulation of reducing sugar directly precedes growth in an organ during the development of seedlings of *M. grandiflora*. TOOLE (43) reports a correlation between the appearance of reducing sugar in a tissue or organ and its decided enlargement. ECKERSON (18), CHOATE (8), PACK (35), and DAVIS (10) report much reducing sugar present in the hypocotyl or coleorhiza just before the pushing out of the hypocotyl. In *M. grandiflora* reducing sugars can be demonstrated in growing regions but never in large amounts. There seems to be no correlation in time of appearance with the swelling or growth of the organ.

The presence and development of the oxidizing systems is worthy of some comment. The presence of catalase in dry seeds and its increase during the preparation for germination and during the process itself are in agreement with the findings of many investigators. There are, however, notable exceptions. The significance to be attached to the catalase activity of the seeds depends entirely on the function assigned to it. If it is even a rough measure of metabolic activity the increase is significant. Catalase is present in both endosperm and embryo of the seed of *M. grandiflora*.

Although the oxygenase component of oxidase was not indicated by microchemical tests on dry seeds or on those with coats split, it did develop before the emergence of the hypocotyl. ECKERSON (17) reports late appearance of oxidase in seeds of *Crataegus*. TOOLE (43) did not find oxidase in seeds or seedlings of maize. ROSE (40) reports an increase in *Tilia* seeds on germination. Peroxidases, on the other hand, are usually considered to be present in seeds.

As oxygenase was found to be lacking in seeds of *Magnolia grandiflora* which had split their seed coats in preparation for germination, and which must have been respiring, tests for glutathione were made. It was absent in dry seeds but present in all later stages of germination, increasing with development, particularly in growing points and in the phloem until the hypocotyl reached the length of 1 cm. Then it was found decreasing. Tests on *Ricinus* seeds gave similar results. The appearance of glutathione in both these seeds on soaking is in line with the observations of VIVARIO and LE CLOUX

given earlier in this paper. Its distribution in both meristem and reserves agrees with KOZŁOWSKI'S observations (28).

The presence of glutathione in a seed when oxygenase is absent is interesting in view of the fact that it has been considered the most important autoxidizable component of animal cells by eminent physiologists. Possibly other seeds reported as lacking oxidase may possess some such oxidation mechanism. It is significant, too, that iron is found throughout the seed, for late investigations on glutathione accept the idea that iron is a necessary part of the glutathione mechanism.

The fact that glutathione was demonstrated in seeds with split coats hints at the mechanism of absorption of O_2 in the Bunzell oxidase determination on seeds in this stage of development when microchemical tests indicated no oxygenase present. It reconciles the Bunzell determination and microchemical ones.

The sequence of changes during the preparation for germination raises many fundamental questions regarding coordinations of the processes occurring, their concatenations and articulations. One such point is the method of depletion of the endosperm. Does the embryo secrete extracellular enzymes, lipases, ereptases, diastases, to which the living endosperm cells are permeable; or are these enzymes preceded by proteolytic ones produced by the embryo which by killing the cells opens them readily to attack by others? Is it possible that the effect of the stimulated embryo is enzymatic in another sense, in that it removes the products of autohydrolyses already going on in the endosperm cells adjoining it and thus allows their continuance? Many endosperms are capable of self-depletion. Oily endosperms are supposed to be particularly so as they are considered to remain alive throughout artificial or natural evacuation. BRUSCHI (5) claims this for the endosperm of *Ricinus* and considers active respiration a necessary condition.

How could the absorption of water accelerate or start these changes in the endosperm? Glutathione appears with the entry of water in the endosperm of *Magnolia grandiflora*. VIVARIO and LE CLOUX consider that in peas it arises from the hydrolysis of polypeptide complexes. The water imbibed may serve to affect the cells in some way so as to bring about the production of glutathione.

Possibly it only modifies the permeability of the cell so that the necessary proteolytic enzyme already present can reach its substrate in the cell. With the production of glutathione, increased respiration is possible if oxygen is available. Do changes in the embryo condition the absorption of water by it, or does the water result as a by-product of increased respiration made possible by the development of the glutathione mechanism?

How do the after-ripening processes in *Magnolia grandiflora* differ from those described for many other seeds? The preparation for germination includes the absorption from the endosperm of a great amount of carbohydrates and products of protein hydrolysis, a process usually reported as taking place in many embryos after the hypocotyl protrudes. This is not strange, for the embryo of this species has very slight reserves and seems slow to attack its oil. The after-ripening includes also a fivefold increase in size of cotyledons, the principal absorbing organs of this embryo.

Summary

1. This paper deals with the behavior of seeds of *Magnolia grandiflora* during preparation for germination and the early stages of the process, with particular reference to the overcoming of delayed germination, to the chemical changes taking place during after-ripening, and to the presence and development of oxidizing mechanisms.

2. Dry storage in the pulp at room temperature seemed almost as advantageous as moist storage of the disinfected seeds at 10° C.

3. Seeds with the fleshy outer coat retained when set to germinate were destroyed by fungi. It was necessary to remove this coat and soak the seeds in CaOCl_2 solution in order to control this situation.

4. The lignified coat delays germination but does not prevent it in 5-20 per cent of the seeds. Alternation of temperature was the only means found of securing consistent germination percentages above the one just mentioned.

5. During the preparation for germination the seeds acquire an increased resistance to fungal attack.

6. The endosperm of a dry seed is composed of thin-walled cells containing protoplasm packed with small oil globules and grains of

protein reserves but no starch. Traces of reducing sugars and soluble proteins can be demonstrated in cells near the embryo.

7. The embryo in a dry seed has oil globules in every cell. It shows no protein granules nor starch grains, but a trace of reducing sugar and short-chain proteins.

8. The seed at the time of splitting of the coat has an embryo twice the dimensions of that of the dry seed, but still very small, being on the average only 2 mm. long and 1 mm. wide, lying in an endosperm 10 mm. long and 4 mm. wide.

9. Before the hypocotyl protrudes the cotyledons grow until they are almost as long as the endosperm within which they lie. Their width increases in the same proportion.

10. The chemical changes taking place between the splitting of the coat and the protrusion of the hypocotyl are in general trend the same as have been reported by others as occurring during after-ripening and germination. After-ripening in the embryo includes absorption of food from the endosperm, a process usually reported as occurring during germination but necessitated earlier in *Magnolia grandiflora* by the slight amount of reserve food in the embryo. During the interval just mentioned the water content increases from 49 to 61 per cent.

11. Oils decrease in amount in the endosperm, the process beginning near the embryo.

12. Soluble proteins increase in endosperm cells near the embryo and in the embryo itself when reserve proteins disappear in these endosperm cells.

13. Starch appears in the cotyledons in striking quantity when the reserves of the endosperm begin disappearing. No detectable amount of oil in the embryo disappears previous to the appearance of the starch.

14. Traces of reducing sugars as well as of soluble proteins occur in the dry seed. Increases in these in the embryo as germination begins cannot be correlated in time with the beginning of rapid growth of these organs.

15. Iron is present in all parts of the seed and abundant in root tips of the seedling.

16. The epidermal cells of the embryo increase in acidity as indicated by depth of color with neutral red; and the contents of endosperm near these cells change from alkaline to acid.

17. Oxidase is not demonstrated by microchemical tests in seeds with the coat barely split, but becomes evident before protrusion of the hypocotyl and increases during later development.

18. Glutathione appears in the seed during soaking in H_2O . It is demonstrable first in the endosperm, then throughout the embryo as germination progresses. It is present previous to development of the oxidase mechanism.

19. Peroxidase and catalase are present in the dry seed, increasing with germination.

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EFFECTS OF SULPHURIC-ACID DELINTING ON COTTON SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 443

AUVAL HESTER BROWN

(WITH FOUR FIGURES)

Introduction

Cotton seeds vary greatly in their rate of germination (11). This fact has been observed by cotton growers for a number of years; but the reasons for their failure to germinate have been the subject of much comment and diversity of opinion (2, 5, 11) among agricultural research workers. Poor germination frequently necessitates the replanting of whole fields several times (11), or else reduces the yield per acre. The consequent tardiness of vegetative growth also makes fruiting difficult (13) in boll-weevil infested areas, and as a result, the yield is decreased materially.

The rôle of the seed coat in delayed germination among certain plants has been recognized (6, 7) for some time. However, few important laboratory studies have been made in connection with the germination of cotton seeds. On the other hand, the experiment stations of the various cotton-producing states and countries have been active in their efforts to secure a uniform rate of germination, and to disinfect the seed coat which carries parasites (5) in the form of fungal spores and bacteria. With the latter object in view, DUGGAR (8) used the sulphuric-acid treatment as a control measure in his efforts to check anthracnose in Alabama as early as 1911. Complete sterilization of the seed coat was reported and the disease was checked so far as external infection was concerned. LEHMAN (10) also used this method of delinting cotton seeds for the same purpose and obtained similar results. Concentrated sulphuric acid was found superior to such solutions as copper sulphate, mercuric chloride, and formaldehyde, since the fuzz on the surface of the seed did not permit the latter solutions to make contact with it, thus a large portion

of the surface was untouched by the reactions of these disinfectants. The difficulty of applying the sulphuric acid to the seed coat made the method impracticable until BROWN (4) began his studies of angular leaf spot on Pima Egyptian cotton. So successful was the treatment as a disinfectant for this seed-borne parasite, that a machine (5) was devised for the cooperative treatment of cotton seeds on a large scale at a very low cost. This improvement over the tedious methods (8) employed by former workers has stimulated its use in regions where long staple cotton is grown and where angular leaf spot is much more prevalent than on upland cotton. Recently the use of concentrated sulphuric acid on upland varieties has proved helpful both as a disinfectant and as an aid to germination. Experiment stations, particularly in Arkansas (9) and South Carolina (3), have employed H_2SO_4 with these purposes in view. The results obtained by the South Carolina station show a marked increase in the yield of cotton per acre in field tests.

This investigation was undertaken with the purpose of making a careful laboratory study of the effects of H_2SO_4 on the germination rate of four short staple and two long staple varieties of upland cotton commonly grown in Oklahoma, and to study the effects of the treatment on the yield in the field tests.

Materials and methods

Samples of six varieties of cotton seeds were obtained from the Oklahoma experiment station in November, 1927. Four of these varieties, Half and Half, Mebane, New Boykin, and Oklahoma Triumph 44, were short staple upland cotton; the other samples, Acala and Delfos, were long staple upland varieties. The seeds were stored in the basement of a well ventilated building which was heated moderately by steam. Laboratory experiments were commenced the following April. Each variety was tested separately to determine the minimum, optimum, and maximum time limit for treatment by concentrated sulphuric acid of specific gravity 1.84. Small samples of the seeds were placed in beakers and about three times their volume of concentrated H_2SO_4 was added. The mixture of seeds and sulphuric acid was stirred continually with a glass rod to insure uniform contact of the delinting agent with the lint, and a Centigrade thermom-

eter was inserted to permit observation of temperature changes during the delinting process. The duration of the H_2SO_4 treatment was varied, six time-intervals being used for each of the six kinds of seeds used, the intervals being 5, 10, 15, 20, 25, and 30 minutes respectively. At the expiration of a given period of treatment, the mass of delinted seeds and sulphuric acid was transferred to a Buchner funnel and the H_2SO_4 drained off rapidly. Tap water was run freely over the seeds and through the funnel to remove the acid from the surfaces of the seeds. This operation was performed quickly, and with excess water, to prevent much rise in temperature resulting from the dilution and hydration of the concentrated acid. The delinted seeds were then washed for a few minutes in $Ca(OH)_2$ to neutralize any adsorbed sulphuric acid which had not been removed by the previous washing. Tests were made with litmus paper after three minutes to make sure that the seeds and solution were no longer acid.

To remove the poor quality of shriveled seed from the material, the washed seeds were placed in a pneumatic trough where the faulty light ones were separated from the heavier ones. The latter were placed between filter papers and dried to constant air-dry weight. Samples treated in this manner were designated lots a, b, c, d, e, and f, depending on the duration of the H_2SO_4 treatment. Fifty seeds from each lot were placed between moist filter papers in appropriate germinators. The control for each variety of cotton seed used consisted of fifty undelinted seeds, referred to in this discussion as lot I. Observations were made daily, and records of the germination rate were kept to determine the effectiveness of the treatment in hastening development of the seedlings. Similar experiments on the same varieties of cotton were also conducted in the botanical laboratory of the University of Chicago during the summer of 1929.

Field tests of the germination rate were made in an open field at McAlester, Oklahoma, and in the University of Chicago greenhouse. Delinted seeds were allowed to dry before planting; and in each instance, undelinted ones of the same variety were used as a check. The greenhouse plots were planted in drills, and also in pots which were sunk in soil. The field tests at McAlester, made in 1931, included a study of the yield from plants derived from delinted and undelinted seeds. The plants were cultivated according to standard

field practices, and harvested at the normal time. The growth and yields are expressed in pounds of cotton produced per acre.

The intake of moisture by cotton seeds was measured during the summer of 1929 on one variety, Oklahoma Triumph 44. Lots of ten seeds each were selected, one with lint on, one with lint removed with sulphuric acid, and one with the brown shell removed from the embryo and endosperm. Each lot was weighed at constant air-dry weight and placed between moist filter paper in germinators. Weighings of each lot were made at the end of 1, 3, 5, and 22 hours respectively to determine the amount of moisture taken in by the cotton seeds under each of the three conditions. Observations were stopped at the end of 22 hours, when the seeds with brown shells removed from the embryo and endosperm had germinated. The percentage of moisture taken in was calculated and recorded.

Results

The delinting process reduced the time required for cotton seeds to germinate in both laboratory and field tests. Results obtained in these experiments varied with the six varieties. Data secured from tests made in the laboratory, in the open field, and in greenhouse plots are given in tabular form. The results with the Acala variety are shown in table I.

The yellow color of the linters on the seeds of the Acala cotton, and the great number of light-weight seeds removed by flotation during washing, indicated low quality seed, and many small inferior seeds were actually found among them. The poor condition of the seed related to poor crop yield, injury in the field owing to climatic factors, or improper storage of seeds after ginning (9). The low percentage of seeds germinating in the control at the end of the sixth day is therefore mainly due to the fact of this inferior quality. The delinting process was probably continued too long for long staple cotton, which was more uniformly delinted by ginning than the short staple varieties used in this work. Injury was observed in lots b to f inclusive, which were subjected to treatments of longer duration than 5 minutes, the limit of the delinting process used in lot a.

Temperatures observed during the delinting process were far below the thermal death point of cotton seeds (11) and apparently were of no consequence in the results obtained.

Seeds of the variety Delfos, also a long staple upland cotton, were of better quality than those of Acala (table I). The data reported in table II show not only a higher percentage of germination in the control, but a definite increase in the rate and percentage of germination of the delinted seeds. Five minutes was found to be the optimum time for treatment in this variety. Although a higher

TABLE I
EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY
SEEDS OF ACALA VARIETY OF COTTON; 6/25/29

Lot	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			3 days	4 days	5 days	6 days
a.....	5	37	6	16	18	48
b.....	10	38	6	18
c.....	15	36	10	12
d.....	20	36	10	10	10	10
e.....	25	34	Attacked by mold			
f.....	30	34	2	4	8	10
i.....	Control	6	18	36

TABLE II
EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS
OF DELFOS VARIETY OF COTTON; 6/2/29

Lot	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a.....	5	35	52	80	88	90
b.....	10	35	30	66	78	80
c.....	15	35	32	66	82	82
d.....	20	38	68	90	92	92
e.....	25	34	48	80	88	90
f.....	30	32	68	78	84	84
i.....	Control	30	68	82

percentage of germination was obtained in lot d, the radicles and seed leaves showed injury by the delinting agent. All lint was removed in 5 minutes, and the surfaces of the seeds were sterilized, which accomplished the purposes of the delinting process. In both of the long staple cottons used, therefore, treatment exceeding 5 minutes is likely to have an injurious effect upon the embryos from penetrating sulphuric acid.

Half and Half variety of cotton produced lint of inferior quality which was easily removed by the delinting process. Optimum duration of treatment by concentrated H_2SO_4 was found to be 5 minutes. Slight injury from the delinting agent was observed in lot d, and this was very pronounced in lots e and f (table III). Pot cultures grown in the greenhouse showed an increase in the rate of germination in lot c (fig. 1, right), which was delinted 15 minutes by concentrated H_2SO_4 ; and also in lot 1 (fig. 1, left), which was the control. The plants were photographed 7 days after planting. The tendency of

TABLE III
EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY
SEEDS OF HALF AND HALF VARIETY OF COTTON; 6/29/29

LOT	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a.....	5	36	74	96	94	100
b.....	10	36	84	92	92	92
c.....	15	36	86	94	94	96
d.....	20	38	76	90	92	92
e.....	25	34	76	80	82	86
f.....	30	36	44	64	80	84
1.....	Control	6	84	98

the undelinted tegmen to inclose the seed leaves after the hypocotyl has raised them above the soil can be seen in figure 1 (left). The sulphuric-acid treatment enables the cotyledons to escape (4) more readily from the investing membranes.

The Mebane sample was in poor condition, probably because of improper methods of harvesting or storing (9). Many faulty shriveled seeds were found when delinted by concentrated H_2SO_4 . A great number of these were removed by flotation in the washing process, but the remainder gave inferior germination because they were severely attacked by fungi when placed in germinators. The fungal attacks were by species of *Rhizopus*, which according to LEHMAN (10) were caused by spores and mycelium that were within the seed coat and could not be reached by surface disinfectant. TOOLE and DRUMMOND (11) attributed the growth of these fungi to the moisture content of the seed. In work done with Texas cotton, it

was found that if the moisture content of stored seed fell below 10 per cent, there was a gradual increase in the tendency of the sample to mold and decay during germination. Fungal growth was more



FIG. 1.—Cotton seedlings, variety Half and Half; A (left), rate of field germination of 50 undelinted seeds; B (right), rate of field germination of 50 delinted seeds. Photographs taken 7 days after planting.

TABLE IV

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF MEBANE VARIETY OF COTTON; 6/27/29

Lot	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			3 days	4 days	5 days	6 days
a.....	5	36	4	8	12	Mold
b.....	10	37	14	14	14	"
c.....	15	37	6	8	16	"
d.....	20	36	6	20	20	"
e.....	25	35	16	16	18	"
f.....	30	33	18	18	20	"
i.....	Control	Attacked by mold			

abundant on the control than on delinted seeds, but was present the sixth day in every lot because conditions for its growth were exaggerated in the germinators.

The optimum time limit for treatment by concentrated H_2SO_4 in the New Boykin variety was 5 minutes. All treatments beyond this

duration showed injury, both to the seed coat, which was pitted and granular and often stained the walls of the germinator a dark brown, and to the radicles which were dwarfed and irregular in shape. The control gave a low percentage of germination caused by the growth of mold probably resulting from spores and mycelium borne in the lint, since the fungus did not occur on the delinted seeds.

TABLE V

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF NEW BOYKIN VARIETY OF COTTON; 6/29/29

Lot	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a.....	5	35	64	78	84	90
b.....	10	35	34	42	48	64
c.....	15	35	54	72	74	82
d.....	20	37	12	12	22	34
e.....	25	35	28	40	54	56
f.....	30	35	28	38	52	52
i.....	Control	4	56	76

TABLE VI

EFFECT OF DELINTING ON PERCENTAGE OF GERMINATION IN FIFTY SEEDS OF OKLAHOMA TRIUMPH 44 VARIETY OF COTTON; 6/22/29

Lot	DELINTED BY CONCENTRATED H_2SO_4 (MINUTES)	TEMPERATURE DURING DELINTING (°C.)	PERCENTAGE GERMINATION			
			2 days	3 days	4 days	5 days
a.....	5	37	82	94	94	94
b.....	10	37	78	86	96	96
c.....	15	42	74	86	94	94
d.....	20	40	96	100	100	100
e.....	25	41	94	100	100	100
f.....	30	41	26	52	62	62
i.....	Control	16	74	94

The optimum duration of treatment by concentrated H_2SO_4 in the Oklahoma Triumph 44 variety was 20 minutes. Figure 2, at the left, shows the appearance of seeds before the process of delinting. Figure 2, at the right, shows the seeds from the same sample which were given treatment for 20 minutes. Figure 3 shows the difference in the rate of germination when both lots had been under germinating con-

ditions for 48 hours. Seeds delinted by sulphuric acid for 10 and 25 minutes respectively also showed that the duration of treatment could be lessened or extended in this variety without influencing the results materially. Lot f, which was given treatment for 30 minutes, showed definite injury to both the seed coats and the radicles of the germinating seeds. In other lots the rate of germination was de-

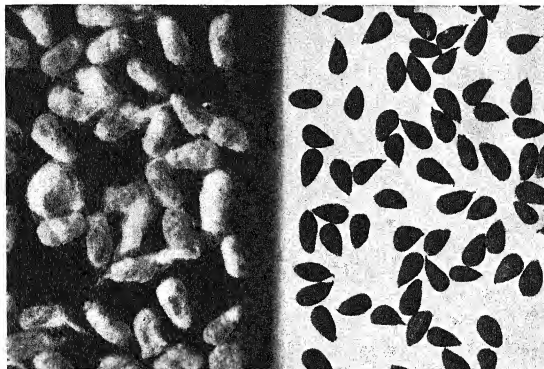


FIG. 2.—Cotton seeds, variety Oklahoma Triumph 44. Left hand side shows appearance of seeds before process of delinting; right hand side shows sample of same seeds after delinting treatment by concentrated sulphuric acid for 20 minutes.

cidedly more rapid in the treated seeds. The germination in delinted seeds was one to three days in advance of the controls.

The test for the effect of delinting upon the yield was conducted on sandy loam soil of average fertility under boll-weevil conditions where early germination is essential. Both plots for delinted and control were planted in rows 150 yards long and 3 feet apart, and the results (table VII) were based on the number of pounds of seed cotton produced per acre. The yields were high for this locality, in which the cotton was grown for the 1931 season. Oklahoma Triumph 44 gave the highest percentage of germination, produced the best fruiting habit, and yielded the highest average of seed cotton

per acre. The rate of germination in the field was based upon the appearance of the cotyledons above the ground. In each plot, delinted seeds germinated two days earlier than the control; and, even until harvest time, both the vegetative growth and the fruiting habit of the seedlings were much better than those from undelinted seeds.

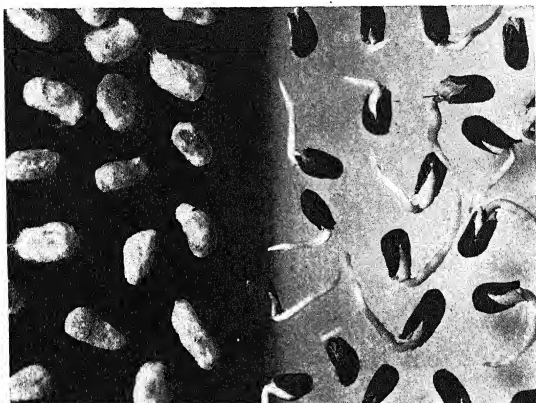


FIG. 3.—Cotton seeds, variety Oklahoma Triumph 44, showing rate of germination of delinted and undelinted seeds. Left hand side of figure shows undelinted seeds under germinating conditions for 48 hours; right hand side shows delinted seeds under germinating conditions for 48 hours.

Further proof of the shorter time required for the germination of delinted seeds is shown by the results of soil tests conducted in the greenhouse. The results shown in table VIII were obtained from a plot of ground in the University of Chicago greenhouse. Counts were made daily from the fourth to the ninth day, counting all seedlings the cotyledons of which had opened, and basing the percentage of germination on the total number of seeds planted. Variations were more noticeable in the varieties Half and Half and Oklahoma Triumph 44. Observations made 15 days after planting showed that

seedlings produced from delinted seeds were more uniform and vigorous than those of the control of the same variety.

Accurate weights, to a thousandth of a gram, showed that the lint was a hindrance to the absorption of water by the seeds. The rapid

TABLE VII

EFFECT OF DELINTING COTTON SEED BY SULPHURIC ACID FOR 5 MINUTES ON
YIELD OF SIX VARIETIES (FIELD TESTS); SEEDS PLANTED 5/10/31

VARIETY	RATE OF GERMINATION (DAYS)		FRUITING HABIT		YIELD IN LB. PER ACRE	
	DELINTED	UN- DELINTED	DELINTED	UN- DELINTED	DELINTED	UN- DELINTED
Acala	3	5	Good	Good	967.80	612.94
Delfos	3	5	"	"	1064.58	838.76
Half and Half	3	5	"	"	1032.32	1029.10
Mebane	3	5	"	"	806.50	677.46
New Boykin	3	5	"	"	1032.32	709.72
Oklahoma Triumph 44	3	5	"	"	1096.84	838.76

TABLE VIII

EFFECT ON RATE AND PERCENTAGE GERMINATION OF DELINTING SIX VARIETIES OF
COTTON SEEDS BY CONCENTRATED H_2SO_4 TREATMENT FOR 20
MINUTES (GREENHOUSE TESTS)

LOT	VARIETY	DATE (1929)	TREAT- MENT	PERCENTAGE GERMINATION (DAYS)					
				4	5	6	7	8	9
a.	Acala	6/25	Delinted	55	85.0	95.0	95.0
b.	Delfos	6/28	"	45	65.0	67.5	85.0
c.	Half and Half	6/28	"	80	85.0	95	95.0	95.0	95.0
d.	Mebane	6/28	"	25	30.0	57.5	80.0
e.	New Boykin	6/28	"	30	55.0	80.0	87.5
f.	Oklahoma Tri- umph 44	6/22	"	50	77.5	90	90.0	90.0	90.0
1.	Acala	6/25	Control	10.0	75.0	90.0
2.	Delfos	6/28	"	25.0	50.0	62.5
3.	Half and Half	6/28	"	45	65.0	67.5	85.0
4.	Mebane	6/28	"	20.0	50.0	60.0
5.	New Boykin	6/28	"	27.5	72.5	80.0
6.	Oklahoma Tri- umph 44	6/22	"	50	67.5	67.5	72.5

increase in absorption by seeds with the brown shell removed from the embryo and endosperm gave a much higher rate of germination than seeds with either smooth seed coats or lint. At the end of 22

hours, seven out of ten seeds with shells removed had germinated; and five out of ten delinted seeds had started to germinate; whereas the seeds with lint were only slightly swollen.

Discussion

The results of this investigation show that delinting cotton seeds by concentrated sulphuric acid gives both a higher percentage and a more rapid rate of germination. The delinted seeds make closer con-

TABLE IX

ABSORPTION OF WATER BY OKLAHOMA TRIUMPH 44 VARIETY OF COTTON SEEDS

SEED TREATMENT	WEIGHT OF 10 SEEDS (GM.)	AMOUNT OF WATER ABSORBED							
		1 HOUR		3 HOURS		5 HOURS		22 HOURS	
		GM.	PER CENT	GM.	PER CENT	GM.	PER CENT	GM.	PER CENT
1. With lint.....	1.2100	0.0312	2.5	0.0534	4.4	0.0721	5.9	0.3846	31.7
2. Lint removed with H ₂ SO ₄	0.9657	0.0390	4.04	0.2710	28.6	0.494	51.1	0.756	78.2
3. Shell removed from embryo...	0.8261	0.2710	20.4	0.4232	51.2	0.5889	71.3	1.1065	133.9

tact with the water of the soil, thus germinating more readily. The intake of water by seeds on filter paper in germinators was much higher in delinted than in undelinted seeds. Exclusion of water by the seed coat was regarded by CROCKER (6) as the real cause of delay in germination of *Axyris amaranthoides* and *Abutilon avicennae*. He thought that the seed coats of many others, as for instance the hard seeds of the Leguminosae and Malvaceae, prevented the embryos from absorbing sufficient water for germination. Slow water absorption seems to be the cause of delayed germination in the undelinted seeds of cotton. The sharp contrast between the rate of germination of treated and untreated seeds of variety Oklahoma Triumph 44 is illustrated by figure 3. The two samples were placed under germinating conditions at the same time. The undelinted seeds are swollen but not germinated, whereas the delinted seeds show 100 per cent germination. Accurate measurements of the absorptive rate of water by these seeds is shown in figure 4.

Field tests show that the seed coat is a hindrance to germination.

The seedlings produced from delinted seeds in the open field and in the greenhouse plots appeared above the soil unhampered by the tegmen. On the other hand, the linted tegmina cling to the soil and prevent the unfolding of the cotyledons. BROWN (4) found that seed coats covered with lint were adapted to holding fast to the soil, in this way delaying germination. Another way in which seedling development was delayed was by the adherence of the tegmen to the cotyledons for several days after they were above the ground. This is illustrated by figure 1, which shows the linted tegmen around the cotyledons, while the right hand side of this same figure shows the increased growth of seedlings from delinted samples of the same variety, Half and Half. Both pots were photographed 7 days after the date of planting. WATKINS (12) reported that cotton seedlings produced from seeds treated with superphosphate paste had difficulty in splitting the tegmen. The treatment had so hardened the covering that, although rooted, the young plants were unable to unfold the seed leaves and make their appearance, and as a result were killed. Sulphuric-acid treatment of seeds makes the seed coat more porous and increases the absorption of water to such an extent that the coat is easily removed by the seed leaves soon after their appearance above the soil. The delinting process can be accomplished by concentrated sulphuric acid without injury to the embryo, provided

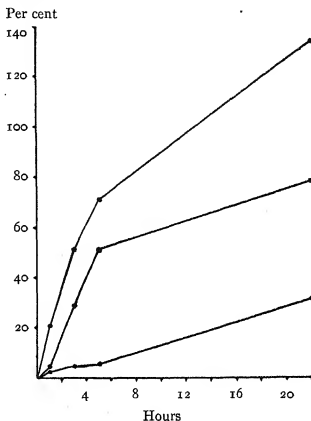


FIG. 4.—Upper graph, absorptive rate of water by cotton seeds with brown shell removed from embryo and endosperm; center graph, same with lint removed with H_2SO_4 ; lower graph, same with lint on seeds.

the treatment is not too prolonged. The optimum duration of treatments by the delinting agent was found to vary slightly with the six varieties of cotton studied. Five minutes was found to be sufficient time to remove the lint and to sterilize the surface of the seed coat. Light shriveled seeds could be detected and removed by flotation after this period; hence for practical purposes this duration of treatment is adequate.

Crude and tedious methods used by early workers (8), and the danger in handling sulphuric acid (5) in open vessels seemed to discredit this form of delinting cotton seeds. When DUGGAR (8) first used the method in Alabama in 1911, the process was slow and only partially destroyed the anthracnose, which was an internal as well as an external parasite. The practice was abandoned until 1922, when interest was renewed (9) to secure more rapid germination and to sort the heavy from the light seeds by flotation. A very practical machine was designed, the initial cost of which can be met by co-operative and extensive use (5). It can be made to serve several purposes; for instance, when grains are disinfected with certain dusts, the machine serves both for dusting and delinting (5).

The method of delinting cotton seeds by sulphuric-acid treatment is superior to that of cutting the lint by oil-mill machinery. In the first place, the removal of the lint is more uniform and much more complete. Seeds of varying size, delinted by saws operated mechanically, are frequently crushed and cut, because no provision is made for the irregularities of form in that method of delinting. The concentrated sulphuric acid reacts with the cellulose of the lint very quickly, and the result is a smooth seed that is entirely intact and with no injury to the embryo. The linters removed by oil-mill machinery (1) may be saved by that process; but their commercial value has depreciated, and they are no longer of importance owing to the inferior quality of the lint so obtained.

The sulphuric-acid treatment produces smooth delinted seeds which can be planted by machine to better advantage than can those with seed coats covered by lint (9, 5). Planters of different types must be used so that the seeds will not be crushed by the revolving plates, for delinted seeds will feed through the machine with as much ease as other smooth seed like corn and beans. The delinted seeds may be spaced accurately with check-row devices. Uniformity

in the rate of germination gives vigorous seedlings which permit early cultivation. This promotes better growth, an important factor in boll-weevil infested areas (13) where early cotton is desirable to increase the yield. The difficulty of cotton planting when the soil is cold from excessive rains can be partially overcome by this treatment, because delinted seeds planted a few days late germinate rapidly and make up for the delay in planting. In this way conditions for early fruiting may be produced to offset losses from the boll weevil.

Summary

1. Tests of the value of delinting the seeds with H_2SO_4 before planting have been made with six varieties of cotton used widely in Oklahoma.
2. Delinting of the seeds by means of this agent gives a higher percentage and an increased rate of germination. No injury to the embryo results, providing the treatment is not prolonged.
3. The successful optimum duration of the delinting process was 5 minutes for long staple upland varieties, and could be extended to 20 minutes without injury in short staple cotton seeds.
4. Yields from mature plants derived from delinted seeds exceeded those from undelinted by an average of 21.4 per cent, under the same field conditions.
5. Smooth delinted seeds are well adapted to agricultural practices with modern machinery; and by the cooperative use of a delinting machine, the cost is moderate.

Grateful acknowledgments are made to DR. CHARLES A. SHULL of the University of Chicago for his helpful criticisms, to L. L. LIGON of the Oklahoma Agricultural Experiment Station for seeds of the six varieties used, and to EDWARD BROWN for assistance in care of field plots.

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FLOWER BUD FORMATION IN THE CONCORD GRAPE

JOHN C. SNYDER

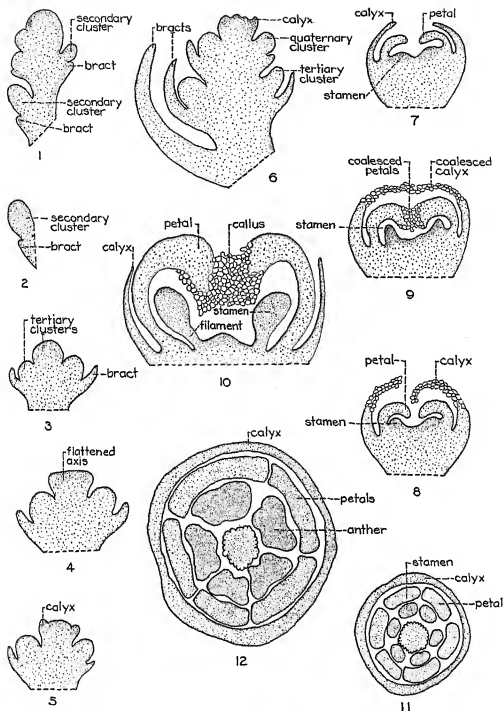
(WITH TWENTY-TWO FIGURES)

Introduction

Most literature concerning the morphology of the flower of the American grape, *Vitis labrusca*, deals with the late stages of development rather than with the origin of the inflorescence. DORSEY (5, 6), BEACH (1, 2), and BOOTH (3) studied the later stages of flower development, and the recent work of PARTRIDGE (10) and COLBY (4) consists primarily of field experiments. Although GOFF (8) states that "embryo flowers" are discernible in the grape bud during the previous season, and JOHNS (9), working with the grape in 1925, reports small "masses of flowers" forming about October 30, there is little detailed information concerning the initiation and early development of the inflorescence. The studies which are presented in the following pages attempt to supply this information.

Materials and methods

In the grape, a group of two to five buds is present at each node, the group being referred to as an eye. Although each bud of an eye may produce a shoot, it is more common for only one shoot to develop at a node. One of the several buds, being larger and structurally more advanced than the others, is referred to as the primary bud and the progressively less developed buds are referred to as secondary, tertiary, and quaternary buds respectively (fig. 21). The primary buds from three to five shoots were examined, being collected at weekly intervals during the growing season and at tri-weekly intervals during the dormant period. Projection drawings were made from longitudinal serial sections of a sufficient number of buds to show their chronological development for the year 1931-1932. The serial arrangement of the buds on the shoots was maintained during each collection, so that comparisons could be made of buds



FIGS. 1-12.—Fig. 1, primordial cluster with secondary clusters subtended by bracts. Fig. 2, secondary cluster and bract removed from cluster shown in fig. 1. Fig. 3, secondary cluster subdivided to form three tertiary clusters. Fig. 4, secondary cluster showing three tertiary clusters (axes of third order), central one showing a flattening, the first evidence of flower formation. Note determinate type of inflorescence of secondary clusters shown in figs. 4-6. Fig. 5, calyx evident in central flower. Fig. 6, secondary cluster similar to that in fig. 3 except that central tertiary cluster has subdivided to form three quaternary clusters. Calyx evident in central quaternary cluster. Fig. 7, flower showing calyx, petals, and stamens. Note that calyx and petals have started to form cap over floral axis. Fig. 8, flower with calyx showing considerable proliferation. Fig. 9, flower with calyx and petals coalesced. Fig. 10, flower with calyx cap ruptured. Petals show marked coalescence and stamens have differentiated into anther and filament. Fig. 11, flower in cross-section showing calyx, petals, and stamens. Fig. 12, flower in cross-section showing calyx and petals, and anthers in quadrilobed condition. $\times 8$.

from one position collected at any one time with buds from other positions and other times of collection. An acetic-formalin-alcohol killing fluid was used. The penetration of the reagents and the sectioning of the buds were greatly facilitated by shaving off one face of each eye and removing the bud scales and hairs. Sectioning was further facilitated by treating the buds with hydrofluoric acid and soaking the paraffin-imbedded specimens in warm water for 1-8 days. Fast-Green dissolved in 95 per cent alcohol proved very satisfactory.

Initiation and development of cluster

Lateral buds arise as protuberances near the tip of a shoot and are inclosed by bud scales. The apical primordium of each bud elongates and in turn produces several lateral outgrowths, the primordia of bud scales, leaves, and inflorescences (fig. 13). By the time there are approximately four such outgrowths on the axis of the bud, two of them, located alternately near the base of the axis, have elongated and become somewhat pointed. These can be identified at this stage as primordial leaves (fig. 13). Additional lateral primordia appear concurrently with elongation of the axis. The distal primordia are produced in pairs, one on either side of the bud axis, differing from those at the base of the axis, which are single. The lobes of each pair, although alike in the initial stage, soon acquire different shapes, one taking on the characteristic shape of a leaf primordium and the other becoming obovate. This obovate primordium is the first evidence of a cluster (fig. 13). While differentiation from leaf to cluster primordium is taking place, axillary buds are being laid down in the axils of the two previously formed primordial leaves (fig. 13).

A diagram (fig. 22) is presented in order to clarify the nomenclature necessary in describing a cluster. The inflorescence of the grape is a racemose panicle. The main axis (*a*) bears lateral axes of the first order (*b*), and these in turn bear lateral axes of the second and third orders (*c*, *d*). The lateral axes arising near the base of the rachis are longer and more profusely branched than those arising from the more distal regions. The degree of branching gradually decreases toward the apex, so that the distal part of the cluster usually consists of simple axes. The cluster of fruit borne by each axis is referred to as a secondary, tertiary, or quaternary cluster, ac-

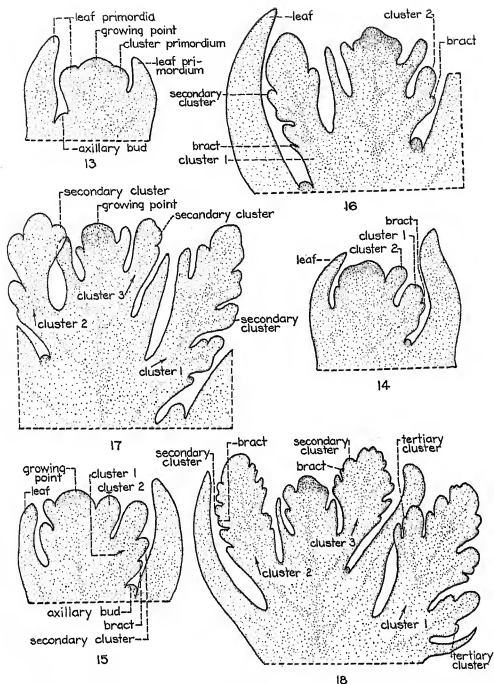
cording to the order of the axis from which it has developed. This of course is not true where the pedicel is attached directly to the rachis, inasmuch as a single fruit can hardly be considered a cluster. On account of the necessity of referring frequently to the subdivisions of the rachis, the term "cluster" is used in preference to axis, "secondary cluster" being more descriptive and convenient, for example, than "axis of the second order."

A cluster bract is first evident in the form of a small lobe, which is sometimes pointed, arising at the base of the primordial cluster (fig. 14). The appearance of an additional lobe on the main cluster marks the initiation of a secondary cluster (figs. 1, 15). The basal secondary cluster seen in figure 1 is detached from the main cluster and shown in figure 2. Each secondary cluster as well as each subsequent subcluster is subtended by a bract (figs. 1, 19, 20). Figure 3 represents the secondary cluster after it has redivided and produced three lobes. Each of these lobes originates directly from the main axis of the secondary cluster and is called a tertiary cluster (fig. 3). One or more of the tertiary clusters may subdivide to form additional lobes which become quaternary clusters. Figure 6 represents a secondary cluster in which the central tertiary cluster has undergone such division. The quaternary clusters in the basal part of the main cluster frequently undergo further subdivision when nutritional conditions are favorable.

Organogeny of flower

The first visible evidence of flower formation is a broadening and flattening of the apex of the floral axis (figs. 4, 19). The calyx appears to be a continuous ring with no indication of separate members, and instead of extending "well up" over the young bud as reported by DORSEY (5), it forms a complete cap over the bud axis (figs. 8, 9, 11). Before the cap is completely formed the petals are evident as definite lobes, and the developing petals ultimately push their way out by rupturing the calyx cap (figs. 7, 10).

As the petals elongate and come nearly into contact with one another, the cells of the apical regions enlarge rapidly, producing a roughened surface. Extensive cell division is initiated in this region, and a considerable mass of callus at the tips of the petals results.



FIGS. 13-18.—Fig. 13, bud representing shoots collected June 5. Note growing point, cluster primordium, leaf primordia, and axillary bud. Fig. 14, bud showing cluster 1 subtended by bract and cluster 2 in form of a simple protuberance, taken from middle region of shoot collected June 13. Fig. 15, bud collected June 20 with cluster 1 showing secondary cluster in axis of bract and cluster 2 in the form of simple protuberance. Growing point and an axillary bud also evident. Fig. 16, bud collected June 27 showing cluster 1 with several secondary clusters. Lobe at base of secondary cluster suggests secondary bract subtending secondary cluster. Fig. 17, bud collected July 17, in which cluster 1 shows numerous secondary clusters. Several secondary clusters are visible in cluster 2, and cluster 3 shows evidence of a secondary cluster. This is the first appearance of a third cluster. Fig. 18, bud collected April 9. In cluster 1 several secondary clusters have produced tertiary clusters. Note that in basal secondary cluster the central tertiary cluster is larger than the other two. Tertiary clusters in this condition frequently undergo further subdivision, producing quaternary clusters. Secondary clusters are numerous in each of clusters 2 and 3. Figs. 13-17 $\times 10$; fig. 18 $\times 20$.

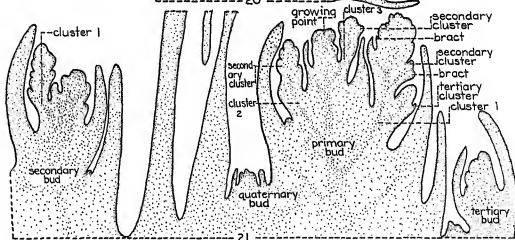
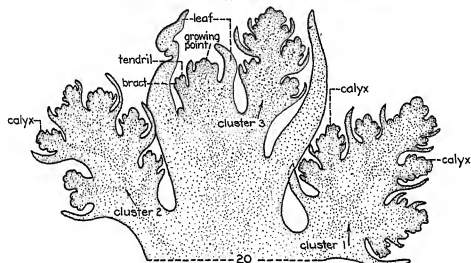
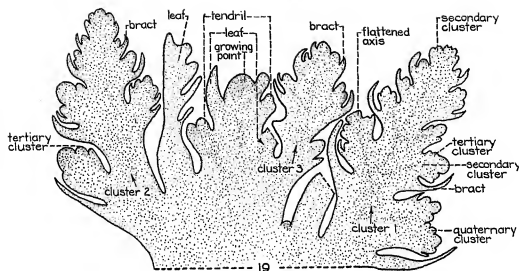
The elongating petals curve inward so that the callus tissues at the tips of opposite petals come into contact and coalesce, thereby bringing about fusion of petals in this region (figs. 9, 10). Adjacent petals, although in contact, apparently do not fuse at the base. The single cycle of stamens, arising inside and opposite the petals, is evident at about the time the petals begin to coalesce (fig. 8). The stamens show no evidence of differentiation into anther and filament at this time, but consist of large irregular cells surrounded by a single layer of small uniform epidermal cells. In cross-section the stamens are obovate, the abaxial surface being nearly in contact with the inner surface of the petals (fig. 11).

Chronological development of cluster on shoot

A flattening of the growing point resulting from extensive cell division of hypodermal cells near one side of the bud axis at this point is the first suggestion of cluster initiation. The cluster, first evident as a shoulder and then as a lateral protuberance from the bud axis, can soon be distinguished from a leaf primordium as described in a previous paragraph (fig. 13). In order to show their chronological development, drawings were made of the most advanced buds from those collections showing more advanced development than buds of preceding collections (figs. 13-21).

The first discernible cluster is evident when the shoot is approximately 1 foot long (fig. 13). Within a week after its differentiation this cluster bears a bract, immediately after which the main part of the cluster undergoes rapid subdivision, forming secondary clusters (figs. 14, 15). Further subdivision of the cluster progresses rapidly until about mid-July, when the growth of both cluster and shoot slows down. The secondary clusters continue to branch, however, so that by the end of the growing season numerous tertiary clusters are discernible in cluster 1.

Cluster 2, evident approximately one week later than cluster 1, develops according to the same plan and at about the same rate (fig. 14). Consequently the second cluster, instead of bearing numerous tertiary clusters at the beginning of the dormant period, bears numerous secondary clusters only (fig. 21). Cluster 3, in comparison with cluster 2, is evident about two weeks later and shows



FIGS. 19-21.—Fig. 19, bud showing first evidence of flower bud differentiation. Note flattened axis in one of basal secondary clusters in cluster 1. In cluster 2 the central tertiary clusters in many of secondary clusters are beginning further subdivision and form quaternary clusters (indicated by increased size compared with adjacent ones). Cluster bracts show considerable development in all three clusters. Tendrils discernible at nodes just beyond cluster 3. This drawing is taken from the 26th bud on a shoot collected April 24. Fig. 20, bud showing first evidence of calyx, the lobes being discernible in clusters 1 and 2. In cluster 3 some of the tertiary clusters are about to undergo further subdivision. Note tendrill with prominent lobes and bract at node beyond cluster 3. This is the 13th bud from shoot collected May 1. Fig. 21, grape eye showing primary, secondary, and quaternary buds, collected September 12. Note presence of several secondary clusters in cluster 3. Fig. 19 $\times 20$; figs. 20, 21 $\times 15$.

evidence of one or two instead of numerous secondary clusters at the beginning of dormancy (fig. 21).

Cluster primordia are initiated in the newly forming buds in the growing region of the shoot simultaneously with development of

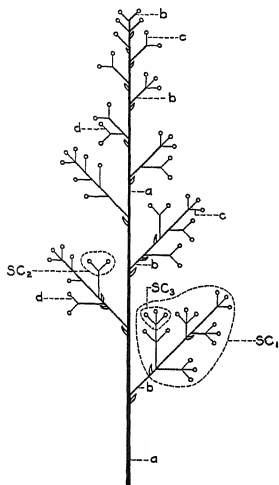


FIG. 22.—Diagrammatic drawing of grape cluster showing: *a*, main axis or rachis; *b*, axis of second order; *c*, axis of third order; *d*, axis of fourth order; *SC*₁, secondary cluster; *SC*₂, tertiary cluster; *SC*₃, quaternary cluster.

the early buds, so that by the end of the growing season all buds on the shoot contain approximately the same number of primordial clusters. Throughout the growing season the buds in the middle region of the shoot show more advanced development than those at its extremities, although toward the end of the season there is an apparent tendency for all buds on a shoot to reach the same stage of development. This condition is realized by the beginning of the dormant period.

The growth of the cluster during the dormant period consists of a very slight enlargement of already existing subclusters. For a period of one or two weeks in the spring, before bud swelling is noticeable, the secondary clusters undergo considerable cell division and en-

largement, resulting in larger and more numerous subclusters. The first visible evidence of flower formation occurs at about the time the buds begin to swell (April 24), appearing first and determinately in the basal subclusters. Differentiation of definite floral structures occurs in acropetal order immediately following the initiation of flower formation.

Summary

The data on flower bud formation in the Concord grape indicate that cluster initiation occurs in the buds of the young shoots and continues in the newly forming buds throughout the growing season. Furthermore, subdivision of the differentiated clusters begins very soon after differentiation of the clusters, and continues until the following spring, when the buds begin to swell and flower formation is evident.

The writer wishes to express his appreciation to those who aided the progress of this investigation, especially Professors B. S. PICKETT, J. E. SASS, and H. W. RICHEY, all of Iowa State College, Ames, Iowa.

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CYTOLOGICAL AND PHYLOGENETIC STUDIES IN THE CUCURBITACEAE

THOMAS W. WHITAKER

(WITH SIXTEEN FIGURES)

Introduction

The majority of investigators working with the Cucurbitaceae have confined themselves to those species which are of economic importance, although MCKAY (12) has reported the chromosome number and behavior of some of the wild species. The present paper submits additional data regarding chromosome number and interspecific and varietal crossing in this group. In addition, the relationships within the family are briefly discussed.

PASSMORE (14) and MCKAY (12) have fully treated the literature. The results of past work on chromosome number and the localities in which the various genera are endemic have been summarized in table I. Because the chromosomes of most members of this group are relatively small and numerous, there has been considerable controversy over the number in many of the species.

The Cucurbitaceae are distributed over most parts of the globe, with the exception of the most extreme sections of the temperate zones and the arctic regions. As a rule they are extremely sensitive to the lower temperatures, and reach their highest development in the tropics of both hemispheres. According to ENGLER and PRANTL, 54 genera are from the Old World tropics and 36 from the New, with 7 genera common to both hemispheres. The recent work of VAVILOV (16, 17) on the origin and distribution of cultivated plants seems to indicate that there are roughly three centers of origin from which the cultivated members of this family have radiated: tropical Africa; the highlands of Central America and southern Mexico; and the Asian center, located along the southwestern slopes of the Himalayas.

Investigation

The seeds for the plants used in this experimental work were secured, for the most part, from various botanic gardens through the

Brooklyn Botanic Garden, the remainder being procured from commercial seed-houses.

The results regarding chromosome numbers of the several species and varieties tested during the course of the work are recorded in

TABLE I

GENUS	CHROMO-SOME NUMBER (n)	HABITAT	INVESTIGATOR
Melothria.....	12	Old World tropics	McKay
Momordica.....	11	Tropical Africa and Asia, 2 species in East Indies	McKay
Luffa.....	11-13	Asiatic tropics	McKay, Passmore
Bryonia.....	10	Mediterranean area	Meurman, Strasburger
Ecballium.....	12	Mediterranean area	McKay
Citrullus.....	11	Tropical Africa	Kozhukhow, Passmore, Whitaker
Cucumis.....	7-12	Old World tropics	Kozhukhow, McKay, Passmore, Heimlich, Whitaker
Bryonopsis.....	12	Africa, East Indies, Aus- tralia	McKay
Benincasa.....	12	Asiatic tropics	McKay
Gymnopetalum.....	11	East Indies	McKay
Lagenaria.....	11	Old World tropics	McKay, Whitaker
Trichosanthes.....	11	Southern Asia, East In- dies, and Australia	McKay
Cucurbita.....	20-24	North and South America	Kozhukhow, McKay, Passmore, Castetter, Ruttle, Whitaker
Coccinia.....	12	Tropical Asia and Africa	McKay
Echinocystis.....	16	North and South America	McKay, Kirkwood
Sicyos.....	12	Probably Europe	McKay
Cyclanthera.....	16	North and South America	McKay

table II. The methods of fixing, staining, and sectioning are the same as those used formerly (18).

In addition to the work on chromosome numbers, the writer was interested in discovering, wherever a reasonable doubt existed, whether there was a possibility of making intergeneric or interspecific crosses in this family, and whether any of these species would cross under field conditions. In an attempt to answer this question, controlled reciprocal pollinations were made according to the scheme outlined in table III.

The varieties of the species used in making the crosses (table III)

were as follows: *Cucumis melo* var. Rocky Ford; *Cucumis sativus* var. Henderson; *Cucumis anguria* var. West India Gherkin; *Citrullus vulgaris* var. Excel; *Cucurbita pepo* var. Long Island Bush; *Cucurbita*

TABLE II

SPECIES	CHROMOSOME PAIRS
Melothria punctata Raf.....	12
M. abyssinica Naud.....	12
Momordica balsamina L.....	11
M. charantia L.....	11
Luffa cylindrica (L.) Roem.....	13
Ecballium elaterium (L.) A. Rich.....	12
Citrullus colocynthis (L.) Schrad.....	11
Cucumis melo L. var. Golden Beauty.....	12
C. melo L. var. Persian.....	12
C. myriocarpus Naud.....	12
Benincasa cerifera Savi.....	12
Cucurbita melanosperma A. Br.....	20
C. pepo var. Orange gourd.....	20
C. pepo Orange gourd×Long Island Bush.....	20
C. moschata var. Calhoun.....	20
Cyclanthera pedata Schrad.....	16

TABLE III

SPECIES	POLLINATIONS	FRUIT SET
Cucumis melo×Cucumis sativus	20	None
Cucumis sativus×Cucumis anguria	20	"
Cucumis melo×Cucumis anguria	20	"
Citrullus vulgaris×Citrullus colocynthis	5*	Three
Citrullus vulgaris×Cucumis anguria	10	None
Citrullus vulgaris×Cucumis melo	10	"
Citrullus vulgaris×Cucumis sativus	10	"
Cucurbita pepo×Cucurbita moschata	20	Two
Cucurbita pepo×Cucurbita maxima	20	None
Cucurbita moschata×Cucurbita maxima	20	Two

* This figure represents the total number of pollinations. Two of the fruit resulted when *C. colocynthis* was used as the pistillate parent; the third fruit had *C. vulgaris* as its pistillate parent.

maxima var. Mammoth Chile; *Cucurbita moschata* vars. Calhoun and Japanese Chirimen.

The technique employed in making the controlled pollinations was as follows. In the case of a typical monoecious species, such as

Cucumis sativus, the staminate and pistillate flowers were isolated on the day previous to anthesis by enveloping the flower in a paper bag. On the following morning the pollinations were made and the pistillate flowers were again inclosed. After several days the bag was removed and the fruit tagged. This procedure was slightly modified for *Cucumis melo*, which is typically andromonoecious, the hermaphroditic flowers being castrated the day previous to anthesis. After this step the procedure was the same as that described for *C. sativus*.

In the three annual species of *Cucurbita*, all of which are monoecious, and in which the flowers are large, each flower was inclosed tightly by means of a rubber band around its corolla tip. The flowers were inclosed on the day previous and pollinated on the morning of anthesis. The female flowers were immediately bagged after pollination, the bag being later removed for tagging.

The results of the work with species crossing in *Cucumis* (table III) indicate that the three species under observation are all cross-sterile. Negative results were also obtained when attempts were made to cross these three species with *Citrullus vulgaris*.

Citrullus vulgaris and *C. colocynthis* are easily hybridized. *C. colocynthis* differs from *C. vulgaris* chiefly in the size of the fruit and seed. The fleshy part of the fruit characteristically has a bitter taste, but possesses the same solidity and color as some forms of *C. vulgaris*. Their vegetative characters are practically identical.

The genetic relationship of the three annual cultivated species of *Cucurbita* has been described by CASTETTER (2) and others. CASTETTER secured hybrid fruit by crossing *Cucurbita pepo* and *C. maxima*. This cross was successful when done reciprocally. In the case of *Cucurbita pepo* × *C. moschata* the situation was somewhat different; fruits were produced only when *C. pepo* was used as the pistillate parent and *C. moschata* as the staminate parent.

The cross *Cucurbita maxima* × *C. moschata* can be made rather easily (table III), and is successful when done reciprocally. An interesting sidelight on this cross is the fact that on the F_1 hybrid plants the staminate flowers are abortive. They are normal in every respect until approximately two or three days before the time of anthesis; then development ceases and the flowers rapidly disintegrate without opening.

During a period of over two years, not a single staminate flower of the F_1 hybrid between *C. maxima* (var. Mammoth Chile) and *C. moschata* (var. Japanese Chirimen) opened, whereas the pistillate flowers were apparently normal although not abundant. Fruit containing viable seeds was secured by back-crossing the pistillate flowers of the hybrid with the staminate flowers of one of its parents (*C. maxima* var. Mammoth Chile). A majority of the pollen mother cells of the staminate flowers of the F_1 plants never undergo the reduction division. They are differentiated from the parenchymatous tissue of the anther, enlarge to some extent, and then become disorganized. Other staminate flowers go through the usual meiotic divisions, and upon formation of tetrads, one (or sometimes two) of the cells of a tetrad fails to develop. The pollen grains of the anther sac have a shriveled appearance.

Of the three fruits obtained from the pollinations between *Cucurbita maxima* and *C. moschata*, two resulted when *C. maxima* was used as the pistillate parent and *C. moschata* as staminate parent. One of these fruits contained viable seed; the other was the result of the reciprocal cross. The seeds of this latter fruit were non-fertile, indicating possibly that the fruit was parthenocarpic in origin. The two fruits resulting from the combination *Cucurbita maxima* \times *C. pepo* contained undeveloped seeds and were probably parthenocarpic.

The results recorded in table III point to the conclusion that species crossing is not a common thing, and, if occurring at all under field conditions, would involve *Cucurbita maxima* and *C. moschata* in *Cucurbita* and *Citrullus vulgaris* and *C. colocynthis* in *Citrullus*.

Discussion

The chief contribution of investigators in the Cucurbitaceae has been the recording of the chromosome numbers of several species. The striking variability of the external morphological characters of a number of the genera and species of this family apparently is not correlated with chromosome number differences. This has been demonstrated in the case of *Cucumis sativus*, in which the chromosome number of a great variety of forms, as reported by several investigators, has always been found to be $n=7$. To determine wheth-

er there was a difference in chromosome number between the ordinary cultivated varieties of this species and some of the aberrant ones, the pollen mother cells of the varieties Lemon and Long Green were examined. In both cases the usual 7 pairs of chromosomes were found (fig. 1).

Similar varietal chromosome number differences are found also in *Cucumis melo*, in which species pollen mother cells have been examined from the ordinary melon (var. Rocky Ford), the Casaba (var. Golden Beauty), and the Persian melon. In each case the haploid chromosome number was 12 (fig. 2). The other species of *Cucumis* examined was *C. myriocarpus*. This species differs widely from *C. melo* in fruit, flower, and vegetative characters. It has 12 chromosomes as the reduced number (fig. 3), which agrees with the counts made from root-tip material by MCKAY (12).

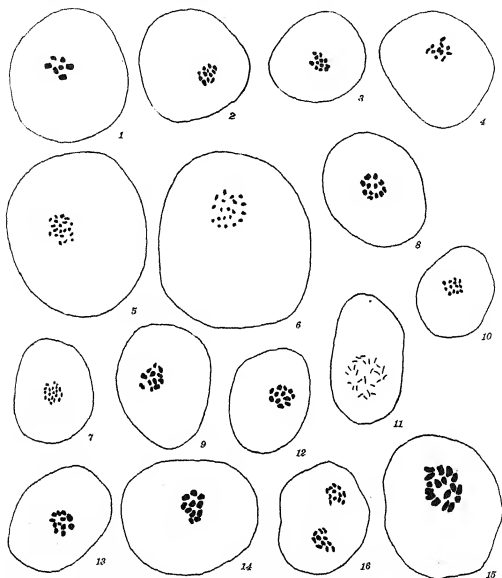
COGNIAUX and HARMS (5) have divided the genus *Citrullus* into four species, *C. vulgaris* Schrad., *C. colocynthis* (L.) Schrad., *C. naudinianus* Hook., and *C. ecirrhosus* Cogn. All these species are endemic in the Central African region. Of these four species, *C. naudinianus* and *C. ecirrhosus* are certainly distinct forms. *C. colocynthis* has generally been regarded as the wild ancestor of the cultivated variety (*C. vulgaris*). According to DE CANDOLLE (6), NAUDIN, in 1838, was able to cross these latter two species and secure fertile hybrids.

The present evidence strongly indicates that *Citrullus colocynthis* is the parent of *C. vulgaris*. Several facts point in this direction: (1) each species has 11 pairs of chromosomes; (2) they can be crossed without difficulty; (3) there is no indication that the hybrids will be sterile; (4) they are endemic in the same locality.

PANGALO (13) has further divided the genera of the Northern Hemisphere into four species: (a) *Citrullus aedulis*, the edible watermelon; (b) *C. colocynthis*, the colocynth; (c) *C. colocynthoides*, the citron melon; and (d) *C. fistulosus*, a small-fruited form from India. It is doubtful whether this practice is of any particular value, as these forms are all of common origin, are cross-fertile, and each has 11 pairs of chromosomes; hence there appears to be no justification for considering them as other than botanical varieties.

The small yellow-flowered gourds belonging to the genus *Cu-*

curbita are undoubtedly a form of *Cucurbita pepo*, as they readily hybridize with any of the forms of the latter and produce fertile hy-



FIGS. 1-16.—Fig. 1, *Cucumis sativus*, 1M. 7 chromosomes; fig. 2, *C. melo* var. Golden Casaba, 1M. 12 chromosomes; fig. 3, *C. myriocarpus*, 1M. 12 chromosomes; fig. 4, *Citrullus colocynthis*, 1M. 11 chromosomes; fig. 5, *Cucurbita pepo* var. Orange gourd, 1M. 20 chromosomes; fig. 6, *C. pepo* var. Orange gourd \times Long Island Bush, 1M. 20 chromosomes; fig. 7, *C. melanosperma*, 1M. 20 chromosomes; fig. 8, *Benincasa hispida*, 1M. 12 chromosomes; fig. 9, *Ecballium daterium*, 1M. 12 chromosomes; fig. 10, *Luffa cylindrica*, 1M. 13 chromosomes; fig. 11, *L. cylindrica*, 26 somatic chromosomes; fig. 12, *Melothria abyssinica*, 1M. 12 chromosomes; fig. 13, *M. punctata*, 1M. 12 chromosomes; fig. 14, *Momordica balsamina*, 1M. 11 chromosomes; fig. 15, *Cyclanthera pedata*, 1M. 16 chromosomes; fig. 16, *Momordica charantia*, 2M. 11 chromosomes; $\times 2400$.

brids. Chromosome counts of the pollen mother cells of the Orange gourd show 20 haploid chromosomes (fig. 5). When this variety

(Orange gourd) is crossed with *C. pepo* var. Long Island Bush, the F_1 has 20 chromosomes (fig. 6).

The plants grown by the writer under the name *Cucurbita melanosperma* A. Br. correspond closely with BAILEY's description (1) of *Cucurbita ficifolia* Bouche. This is the Malabar, or fig-leaved gourd, and it has 20 chromosomes as the reduced number (fig. 7).

The fruit, seed, and vegetative characters of the plants grown under the name *Benincasa hispida* Thunb. were identical with those figured by COGNIAUX and HARMS (5). These investigators give *B. cerifera* Savi. as a synonym for *B. hispida* Thunberg. This is the so-called white gourd, or wax gourd, cultivated for its edible fruits in China and India. The haploid chromosome number of the plants of this species was found to be 12 (fig. 8), which agrees with the chromosome number of the plants grown by MCKAY (12) under this name (*B. hispida*). The plants grown by MCKAY, and labeled *B. cerifera*, are most probably a species of *Cucurbita*, since they had 42 chromosomes.

The monotypic species *Ecballium elaterium* has 12 haploid chromosomes. The chromosomes of this species are similar to those of other species of the 24-chromosome group of the family, in that they are small and morphologically uniform.

PASSMORE (14), in a study of microsporogenesis in *Luffa cylindrica*, has figured this species with 11 pairs of chromosomes. MCKAY (11, 12) gives *L. marylandica*, *L. gigantea*, and *L. acutangula* with 13 as the haploid number and 26 as the 2n number. *L. marylandica* and *L. gigantea* are synonyms for *L. cylindrica* (COGNIAUX and HARMS 5). It is obvious that these two workers disagree on the chromosome number of the same species, *L. cylindrica*. Plants grown under the names *L. cylindrica*, *L. foetida*, and "Sooly Qua" were similar and, from all indications, belonged to the same species. *L. foetida* is a synonym for *L. cylindrica* (COGNIAUX and HARMS), while Sooly Qua is the Chinese name for the fruit of this species (STURTEVANT 9). All these plants had 13 pairs of chromosomes (fig. 10). Counts of the root-tip material of *L. cylindrica* indicate that there are 26 somatic chromosomes (fig. 11).

Plants of *Melothria punctata* Cogn. and *M. abyssinica* were identical, from all external appearances, so far as the writer's observations were concerned. COGNIAUX (4) gives *M. abyssinica* as a synonym for

M. punctata. The chromosome number was the same in both species, each having 12 as the reduced number (figs. 12, 13).

Momordica balsamina was found to have 11 haploid chromosomes (fig. 14). *M. charantia* was also found to have 11 chromosomes (fig. 16). *Cyclanthera pedata* has 16 chromosomes as the reduced number (fig. 15). The chromosomes of the latter are the largest and show the greatest degree of morphological distinction of any member of the group studied.

In dealing with the phylogeny of this family, there are a few points which it seems desirable to mention. The species that have been cultivated for their food products were all originally confined to three rather restricted areas. The regions and the species native to them are as follows: (1) tropical Africa (*Citrullus vulgaris*, *Cucumis melo*, and *Cucumis anguria*); (2) tropical India (*Luffa acutangula*, *Luffa cylindrica*, *Benincasa hispida*, *Lagenaria vulgaris*, and *Cucumis sativus*; in addition, *Cucumis melo* is found in this region); and (3) the highlands of Central America and southern Mexico (*Cucurbita pepo*, *Cucurbita moschata*, and *Cucurbita maxima*). The Old World species have basic chromosome numbers of 10, 11, 12, and 13 (with one exception, *Cucumis sativus*, which has 7 pairs of chromosomes). The *Cucurbita* species endemic in the New World have 20-24 as the basic number.

The non-cultivated members of this group coincide very closely with the cultivated members. The Old World genera have basic numbers of 10, 12, and 13 chromosomes and the non-cultivated *Cucurbita* of the New World have 20 as the basic number. In addition, there appears a New World group having 16 as the basic number, represented by *Echinocystis* and *Cyclanthera*. *Sicyos angulatus*, which has 12 as the basic number, may possibly be of American origin, but it is considered doubtful by taxonomists.

Taking into consideration the chromosome variability of the genera of the Cucurbitaceae in which the chromosome number for two or more species has been reported, we find that they apparently fall into three groups: (1) The genera in which the chromosome number of all recorded species is the same. In this classification come *Bryonia*, *Luffa*, *Momordica*, *Citrullus*, and *Cyclanthera*. In this group morphological differences apparently are not correlated with chromosome number differences. (2) *Cucumis* is placed in the second

group. There is some evidence that LEVITSKY's theory of phylogeny by chromosome fragmentation, as suggested by KOZHUKHOW (10), may apply to this genus. This suggestion is based on the fact that the 14 diploid chromosomes of *C. sativus* are large, compared with other members of the genus, with several showing median constrictions. These may have fragmented to form the 22 and 24 chromosome members of the genus. The evidence, however, is far from conclusive. (3) *Cucurbita* is placed in the last group. It is an example of a genus showing aneuploidy. RUTTLE (15), however, would place *Cucurbita* in the first grouping, as she is of the opinion that the chromosome number of all species of *Cucurbita* is 40. Several investigators have reported the diploid number of *C. moschata* as 48. Repeating their work, and in some cases using the same varieties of the latter species, RUTTLE finds 20 pairs of chromosomes.

Summary

1. The chromosome numbers of 12 species of the Cucurbitaceae have been determined from pollen mother cell material. The results may be summarized as follows: *Melothria punctata*, $n=12$; *Melothria abyssinica*, $n=12$; *Momordica balsamina*, $n=11$; *Luffa cylindrica*, $n=13$; *Ecballium elaterium*, $n=12$; *Citrullus colocynthis*, $n=11$; *Cucumis myriocarpus*, $n=12$; *Cucumis melo* var. Golden Beauty, $n=12$; *Benincasa cerifera*, $n=12$; *Cucurbita melanosperma*, $n=20$; *Cucurbita pepo* var. Orange gourd, $n=20$; *Cyclanthera pedata*, $n=16$.

2. The results of an experimental study of the fertility relations involving 4 genera and 8 species of the Cucurbitaceae indicate that species crossing is comparatively rare and not at all in accord with popular beliefs. Three species crosses were obtained: (1) between *Cucurbita moschata* and *C. maxima*; (2) between *Cucurbita pepo* and *C. moschata*; and (3) between *Citrullus vulgaris* and *C. colocynthis*.

3. The technique of making controlled pollinations among members of this family has been described.

4. The pertinent points in regard to chromosome number and geographical distribution are: (a) the Old World genera, with one exception, have basic numbers of 11-13; (b) there is a New World group having 16 as the basic number; and (c) there is a second New World group having 20-24 as the basic number.

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MYCORRHIZAS OF TREES AND SHRUBS

LEROY K. HENRY

(WITH SIX FIGURES)

Introduction

The purpose of this investigation has been to determine the extent of mycorrhizal infection of trees and shrubs growing in both woodland and open fields in a limited area in Butler County, Pennsylvania.

METHODS.—The smaller rootlets were carefully uncovered by means of a trowel and then traced to their respective tree or shrub by means of a spade and mattock. It is very necessary in this work to be certain of the plant from which the collection is made, and this can be accomplished only by tracing the rootlets to their point of origin from the larger lateral roots. The coralloid clusters or the swollen tips of rootlets were removed, washed in water, and placed in small bottles containing either Gilson's fixative or 5 per cent formaldehyde.

The usual laboratory methods of dehydrating, clearing, and imbedding in paraffin were employed. Sections were cut 10μ thick, stained in Biebrich scarlet and methyl violet 6B, and mounted in balsam for permanent records. Mycorrhizas were not found on every rootlet of a collection, so that often several had to be examined and sometimes a new collection of roots from the same plant had to be made the following season.

One can be rather certain of the coralloid ectotrophic types by aid of a hand lens, and even of the beaded forms of the endotrophic. In cases of uncertainty, however, it has been found that by soaking the roots in 5 per cent sodium hydroxide for three or four days and then examining under the low power of the microscope, the presence of the mycorrhizas can be determined. After such treatment the ectotrophic types are readily seen as an interwoven, fungal mantle around each rootlet; but the endotrophic types show merely as small beadlike swellings. In order to determine whether these swellings

contained mycorrhizal hyphae, some that had been treated with 5 per cent sodium hydroxide were imbedded. After sectioning and staining, the intracellular hyphae and granular masses were plainly visible.

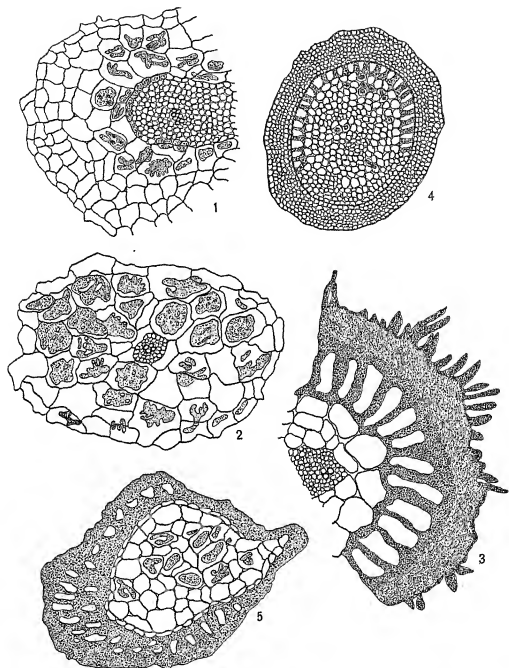
The pH value of the soil around each tree and shrub was determined by the La Motte Soil Teskit. Some rootlets were collected each month from September to June from plants growing in the woods, fields, fence rows, and yard. Mycorrhizas were found on some of the collections made each month, so that it would seem that they occur throughout the year and are not restricted to woodland soils. The mycorrhizas seemed most plentiful during the late fall and early winter, although good slides were often obtained from spring collections. In the winter months many dried and shriveled mycorrhizas were seen, but there were always some that remained in the normal, active condition.

Types of mycorrhizas investigated

Roots were collected from 60 different trees and shrubs growing in four habitats: woods, fields, fence rows, and a yard. In the districts where these mycorrhizal collections were obtained, loamy, silty soils prevail which showed a pH of 5.0 beneath all of the trees and shrubs investigated. Three general types of mycorrhizas were found: endotrophic, ectotrophic, and ectendotrophic. These three types were not confined to any specific kinds of soils, since they all appeared upon plants from all four of the habitats.

In endotrophic infection the hyphae penetrate the root through the root hairs and assume various intracellular forms. Individual hyphae may lie scattered or twisted about one another within the individual cell (fig. 1), or the hyphae may be woven into a skein until they appear like a mass of threads. Often the hyphae are broken into short fragments, or have the appearance of large granular intracellular masses (fig. 2) which, according to various workers as reported by RAYNER,¹ represent hyphae that are being digested by the host cells. The individual hyphae are not strictly confined to any one cell, but often extend from one root cell to another. Ex-

¹ RAYNER, M. C., *Mycorrhiza: An account of non-pathogenic infection by fungi in vascular plants and bryophytes*. New Phytol. Reprint no. 15. Wheldon and Wesley Ltd. London, England. 1927.



FIGS. 1-5.—Fig. 1, cross-section of endotrophic mycorrhiza from *Vaccinium vacillans* showing individual hyphae within the cells; $\times 120$. Fig. 2, cross-section of endotrophic mycorrhiza from *Crataegus macrosperma* showing cortical cells filled with fragments of hyphae and granular masses; $\times 120$. Fig. 3, portion of cross-section of ectotrophic mycorrhiza from *Cornus paniculata* showing prosenchyma tissue; separate hyphae project from outer surface; continuation of fungal tissue produces radial elongated cells in the cortex from inner surface; $\times 340$. Fig. 4, cross-section of ectotrophic mycorrhiza from *Pyrus coronaria* showing pseudoparenchymatous fungal mantle; $\times 120$. Fig. 5, cross-section of mycorrhiza from *Cornus paniculata* showing both ectotrophic and endotrophic infection; $\times 120$.

ternally these infected roots are swollen at the tips and branch dichotomously, or they may appear to have beadlike constrictions borne singly or in a series.

In ectotrophic infection, the hyphae penetrate the root through the epidermis and advance intercellularly until they form a network around the cortical cells. Because of the thick mycelia between the cortical cells, the latter often become radially rectangular in shape and a thick fungal mantle replaces the epidermis (fig. 3). Often the hyphae of the fungal mantle lose their individuality and become merged into a pseudoparenchymatous tissue (fig. 4). The ectotrophic type of infection seldom progresses beyond the endodermis of the root. Roots infected with ectotrophic mycorrhizas assume different external forms. There may be groups of many short roots at the tip of a larger one, giving the appearance of a cluster of corals, whence the term coralloid for this form (fig. 6); or small tubercle-like projections may be found which, when sectioned, show several roots held together by mycelia. This latter form is called tubercular. Since the formation of the fungal mat around the root prevents further growth in length, lateral roots develop which in turn are infected. It is this process that produces the coralloid clusters of ectotrophic mycorrhizas.

These two types of infection are not, as was thought by an early worker as reported by RAYNER, specific upon a particular root. Often one root may show endotrophic mycorrhizas and another root from the same plant may possess the ectotrophic type, or both types may be found upon the same rootlet (fig. 5). It is to this type of infection that the name ectendotrophic mycorrhiza has been given. The external appearance of these roots depends entirely upon the type of their internal infection.

A transition from the endotrophic to the ectotrophic condition was observed in the case of mycorrhizas from *Crataegus macrocarpa*. Collections made in May showed endotrophic infection, but in October the mycorrhizas were ectotrophic in external appearance. When these were sectioned and stained, many granular masses were seen within the cortical cells.

That infection is not localized at any one place on the rootlet, but arises wherever the germinating fungal spore and the root happen to come in contact, was shown by sections of mycorrhizas of both types

cut in a series from the tip to 5 mm. behind it. In the ectotrophic type the mycelia occurred at the tip of the root in the form of a fungal mantle around the outside, while 2 mm. from the tip hyphae began penetrating between the cortical cells, forming the characteristic radially elongate cells.

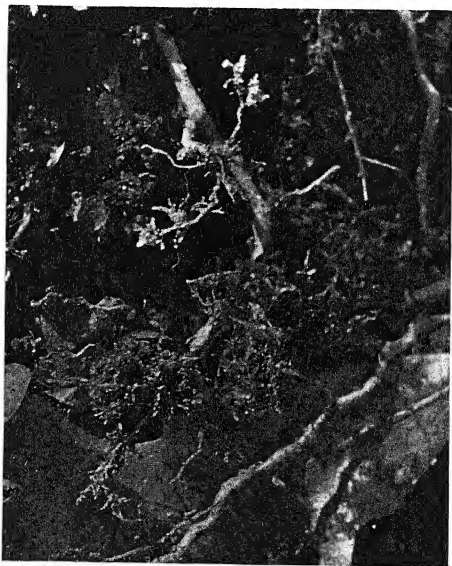


FIG. 6.—Coralloid form of ectotrophic mycorrhiza on lateral roots of *Quercus alba*; slightly enlarged.

The following list of trees and shrubs is grouped, first, according to the location of the tree with regard to ecological habitat; second, according to the types of mycorrhizas found. So far as is known by the writer, the species indicated by an asterisk are new additions to those already identified as mycorrhizal hosts.

Woodland

ENDOTROPHIC TYPE

- | | |
|---|---------------------------------------|
| * <i>Amelanchier oblongifolia</i> (T. & G.) Roem. | <i>Magnolia acuminata</i> L. |
| * <i>Azalea nudiflora</i> (L.) Torr. | * <i>Nyssa sylvatica</i> Marsh. |
| * <i>Benzoin aestivale</i> (L.) Nees. | <i>Pinus resinosa</i> Ait. |
| <i>Carya cordiformis</i> (Wang.) K. Koch | <i>Pinus virginiana</i> Mill. |
| * <i>Fraxinus nigra</i> Marsh. | * <i>Populus grandidentata</i> Michx. |
| * <i>Hydrangea arborescens</i> L. | <i>Rhus glabra</i> L. |
| <i>Juglans cinerea</i> L. | <i>Tilia americana</i> L. |
| | <i>Ulmus fulva</i> Michx. |
| | * <i>Vaccinium vacillans</i> Kalm. |

ECTOTROPHIC TYPE

- | | |
|--|--|
| <i>Alnus rugosa</i> (DuRoi) Spreng. | * <i>Diervilla lonicera</i> Mill. |
| <i>Carpinus caroliniana</i> Walt. | * <i>Hamamelis virginiana</i> L. |
| * <i>Carya alba</i> (L.) K. Koch | <i>Ostrya virginiana</i> (Mill.) K. Koch |
| <i>Carya glabra</i> (Mill.) Spach. | <i>Pinus strobus</i> L. |
| <i>Carya ovata</i> (Mill.) K. Koch | <i>Quercus alba</i> L. |
| <i>Castanea dentata</i> (Marsh.) Borkh. | * <i>Quercus coccinea</i> Muench. |
| <i>Corylus americana</i> Walt. | <i>Quercus velutina</i> Lam. |
| * <i>Crataegus crus-galli</i> L. | <i>Vaccinium stamineum</i> L. |
| * <i>Crataegus pruinosa</i> (Wendl.) C. Koch | <i>Ulmus americana</i> L. |

ECTENDOTROPHIC TYPE

- | | |
|-------------------------------------|--------------------------------------|
| * <i>Cornus paniculata</i> L'Her. | * <i>Pyrus coronaria</i> L. |
| * <i>Crataegus macrosperma</i> Ashe | <i>Quercus rubra</i> L. ² |
| <i>Fagus grandifolia</i> Ehrh. | * <i>Viburnum acerifolium</i> L. |

²In the fall of the year, fungi had been observed growing under trees on which mycorrhizas had previously been found. Removal of excess soil showed mycorrhizal rootlets matted among the dense mycelia at the base of the fungal stipe. After washing thoroughly in water and placing in a fixative, the roots still remained attached to the mycelial mass. Macroscopical investigation would seem to indicate that the fruit body of the fungus was produced by the same hyphae that form the mycorrhiza. In order to verify this assumption, the base of the stipe of *Clitocybe candicans* Pers. from *Quercus rubra*, together with some of its mycorrhizal rootlets, were imbedded, sectioned, and stained. These rootlets showed ectotrophic infection and the cross-sections of the stipe contained sections of roots among the mycelia.

Field**ENDOTROPHIC TYPE**

Gleditsia triacanthos L.	*Rhus typhina L.
Juglans nigra L.	Salix nigra Marsh.
*Morus alba L.	Sassafras variifolium (Salisb.)
Morus rubra L.	Ktze.

ECTOTROPHIC TYPE

Populus tremuloides Michx.	*Salix alba var. vitellina (L.)
	Koch

ECTENDOTROPHIC TYPE

Robinia pseudo-acacia L.

Fence row**ENDOTROPHIC TYPE**

Acer rubrum L.	Liriodendron tulipifera L.
*Cornus florida L.	*Prunus serotina Ehrh.

ECTOTROPHIC TYPE

Fraxinus americana L.

Yard**ENDOTROPHIC TYPE**

*Acer platanoides L.	*Populus alba L.
Acer saccharinum L.	*Sorbus americana (Marsh.) DC.
Picea abies (L.) Karst.	

The majority of endotrophic types from plants growing in fields and in the yard possessed the typical beadlike swellings which contained fragments of hyphae and granular masses. There was an abundance of root hairs upon the normal rootlets, while among the collections from *Acer platanoides*, *Juglans nigra*, *Populus alba*, and *Sorbus americana* comparatively few roots were infected. Not all of the roots of those plants that possessed ectotrophic mycorrhizas were infected, and those that were not had functional root hairs. Thus it would appear that the ectotrophic mycorrhizas took the place of the root hairs, and by producing numerous coraloid clusters perhaps increased the effective absorptive area of the trees. The type, the external form, and the microscopical structure of mycorrhizas on 26 new host plants are described in table I.

TABLE I
DATA FOR NEW MYCORRHIZAL HOST PLANTS

NEW HOST PLANTS	DATE OF COL- LECTION (1930)	HABITAT	EXTERNAL FORM OF INFECTED ROOTS	REMARKS
Endotrophic:				
<i>Acer platanoides</i>	May, Sept.	Y*	Beadlike swellings and swollen tips	Few roots infected
<i>Amelanchier oblongifolia</i>	May, Oct.	W	Swollen tips	Some sporelike bodies and granular masses within cortical cells
<i>Azalea nudiflora</i>	Feb., Oct.	W	"	Granular, intracellular masses and sporelike structures
<i>Benzoin aestivale</i>	Sept.	W	"	Granular, intracellular masses and sporelike structures
<i>Cornus florida</i>	Feb., Oct.	FR	Beadlike swellings and swollen tips	Cortical cells containing granular masses and individual hyphae
<i>Fraxinus nigra</i>	Sept.	W	Swollen tips	Groups of hyphal fragments
<i>Hydrangea aborescens</i>	Sept.	W	"	Granular structures and sporelike bodies in cortical cells
<i>Morus alba</i>	May, Oct.	F	Beadlike swellings and swollen tips	Individual hyphae and hyphae in cross-section
<i>Nyssa sylvatica</i>	May	W	Beadlike swellings	Individual hyphae often extending from cell to cell
<i>Populus alba</i>	May, and Feb. (1931)	Y	Beadlike swellings and swollen tips	Majority of roots normal
<i>Populus grandidentata</i>	May	W	Swollen tips	Broken and individual hyphae
<i>Prunus serotina</i>	Feb., Oct.	R	Beadlike swellings	Intracellular hyphae and vesicle
<i>Rhus typhina</i>	Sept.	F	Swollen tips	Intracellular granular masses and broken hyphae
<i>Sorbus americana</i>	May, Nov.	Y	Beadlike swellings and swollen tips	Majority of roots normal
<i>Vaccinium vacillans</i>	Feb.	W	Dichotomously branched with swollen tips	Root hairs on epidermis; intracellular hyphae

* Y, yard; W, woods; FR, fence row; F, field.

TABLE 1—Continued

NEW HOST PLANTS	DATE OF COL- LECTION (1930)	HABITAT	EXTERNAL FORM OF INFECTED ROOTS	REMARKS
Ectotrophic: <i>Carya alba</i>	March	W	Coralloid	Pseudoparenchymatous fungal mantle; one row of radially elongated cells; endodermis filled with dark-staining material
<i>Crataegus crus-galli</i>	May, and Sept. (1931)	W	"	Fungal mantle with hyphae projecting from periphery; radially elongated cortical cells
<i>Crataegus pruinosa</i>	Sept. (1931)	W	"	Pseudoparenchymatous fungal mantle with radially elongated cells on one side and 3 or 4 irregular rows of cortical cells on other
<i>Diervilla lonicera</i>	Feb. (1931)	W	"	Few roots infected; fungal strands extending between cortical cells
<i>Hamamelis virginiana</i>	Oct.	W	"	Pseudoparenchymatous fungal mantle with radially elongated cells on one side only
<i>Quercus coccinea</i>	Feb.	W	"	Fungal mantle of interwoven hyphae; endodermis filled with dark-staining material
<i>Salix alba</i> var. <i>vitellina</i>	May	F	"	Fungal mantle of interwoven hyphae and strands between outer cortical cells
Ectendotrophic: <i>Cornus paniculata</i>	May, and Sept. (1931)	W	Coralloid and swollen tips	Fungal mantles with hyphae projecting from periphery and radially elongated cortical cells; also intracellular hyphae
<i>Crataegus macrosperma</i>	May, Nov.	W	Coralloid and swollen tips	Pseudoparenchymatous fungal mantles and radially elongated cortical cells; intracellular granular masses and individual hyphae
<i>Pyrus coronaria</i>	Oct., and Sept. (1931)	W	Coralloid and swollen tips	Thick pseudoparenchymatous fungal mantles with strands between cortical cells; intracellular granular masses
<i>Viburnum acerifolium</i>	May, Sept.	W	Coralloid and swollen tips	Narrow fungal mantles of interwoven hyphae; granular masses in cortical cells

Collections made in February, 1930, from a young tree of *Quercus coccinea* growing in the woods, showed ectotrophic infection. In cross-section the younger roots had fungal mantles of interwoven hyphae and radially elongated cortical cells; while the older roots had large, irregular, cortical cells with thin walls, and the endodermis was filled with a dark-staining material. Since this material was absent from the younger roots, it may be that the older roots are attempting to throw off this infection by the production of a substance which prevents the fungus from advancing. The large, thin-walled cortical cells seem to indicate a reversion to a primitive condition as a result of the infection.

Cross-sections of roots of *Hydrangea arborescens* collected in September, 1930, showed endotrophic infection with granular structures and sporelike bodies within the cortical cells. Nuclei were easily seen in those cells containing the spores, indicating that the root cells were still alive when collected.

Summary

Mycorrhizas of three types, ectotrophic, endotrophic, and ectendotrophic, have been discovered on 60 different trees and shrubs, 26 of which are new additions to the list of mycorrhizal host plants.

CARNEGIE MUSEUM
PITTSBURGH, PA.

PHYSIOLOGICAL VARIATIONS IN CERTAIN CROP
PLANTS FOLLOWING SEED EXPOSURE TO
HIGH-VOLTAGE X-RAYS

C. N. MOORE AND C. P. HASKINS

(WITH SIX FIGURES)

In the course of a series of investigations now in progress on the biological effects of x-rays, it was considered of interest to conduct some experiments in the subjecting of dry and moist, but ungerminated, seeds of a number of crop plants to x-rays. Considerable work has been done in this field, notably by ANCEL, GOODSPEED, and STADLER,¹ but it is noticeable that in most of it the radiation used was distinctly soft, the source of radiation employed in most cases being a Coolidge tungsten-target air-cooled tube operated at 50 KV and 2 to 5 MA.

It has been thought worth while, therefore, to collect certain cases of physiological abnormalities which were observed in plants arising from seeds exposed to harder radiation, both because of their striking resemblance to certain deformities occurring in *Nicotiana* species exposed as seeds to the softer rays, as reported by GOODSPEED, and because certain species have not, to the knowledge of the writers, been previously subjected to x-rays, either as to sex cells, growing points, or meristematic tissues. The work was conducted entirely from the physiological standpoint, and no histological examination of resulting abnormalities was made, although it is planned to conduct such work on more promising types. Consequently, although it seems highly probable that abnormal mitoses, correlated with considerable genetic changes, are involved, no account can be given of them at the moment. It is difficult to fix the lethal dose for unger-

¹ ANCEL, P., Action des faibles doses de rayons x sur des graines sèches. *Compt. Rend. Soc. Biol.* 91:1435-1436. 1924.

GOODSPEED, T. H., Effects of x-rays and radium on species of the genus *Nicotiana*. *Jour. Heredity* 20:243-260. 1929.

STADLER, L. J., Some genetic effects of x-rays in plants. *Jour. Heredity* 21:3-19. 1930.

minated seeds at any voltage, and this has not been done sufficiently clearly to be reported at this time. It is known, however, that the dosages reported here were well within the limit of tolerance in each case. For this reason, and because of scattering and differential absorption effects, it has not been possible to correlate effects with quantity of incident energy.

SOURCE OF RADIATION.—A standard Victor deep-therapy x-ray outfit was used as the source of radiation, consisting of a high-tension transformer with Snook cross-arm mechanical rectifier and a standard Coolidge water-cooled tungsten-target tube of the thick-walled type. The voltage wave form delivered by the generator was very nearly sinusoidal.

The tube was operated at 200 kilovolts peak, with 30 milliamperes current. The focal distance at which the seeds were exposed was 50 cm. The shortest wave length theoretically obtainable at such a voltage is 0.062 Ångströms. No metal filter was used, but the glass of the thick-walled tube interposes a filtering action equivalent to about 0.10 mm. of copper. At 0.70 Å. the intensity of emergent radiation from the tube is only about 0.03 per cent of that incident on the inner surface, and this may be considered the longest wave length present in the primary radiation. The greatest intensity of radiation lay in the neighborhood of the $K_{\alpha 1}$ line of tungsten, at about 0.21 A.U. The $K_{\beta 1}$ line, at about 0.18 Å., also figures prominently, and general radiation at this voltage is very considerable. Under the conditions of irradiation used, the incident dosage totaled 300 roentgen units per minute, as measured by a Victoreen dosimeter calibrated against a radium-compensated Failla ionization chamber.

TREATMENT OF SEEDS.—The seeds to be treated were divided into two portions, one of which was subjected to several hours of soaking in distilled water, followed by superficial drying with filter paper, before irradiation. Since no difference of susceptibility could be found between the dry and moist seeds at this voltage, and since this had been a usual experience with other workers using softer radiation, the distinction proved an artificial one and was eventually abandoned. Both seed lots were then spread as a single layer in containers on a table beneath the tube and given treatments varying from 0.5 to 128 minutes. Immediately thereafter the seeds were sowed in

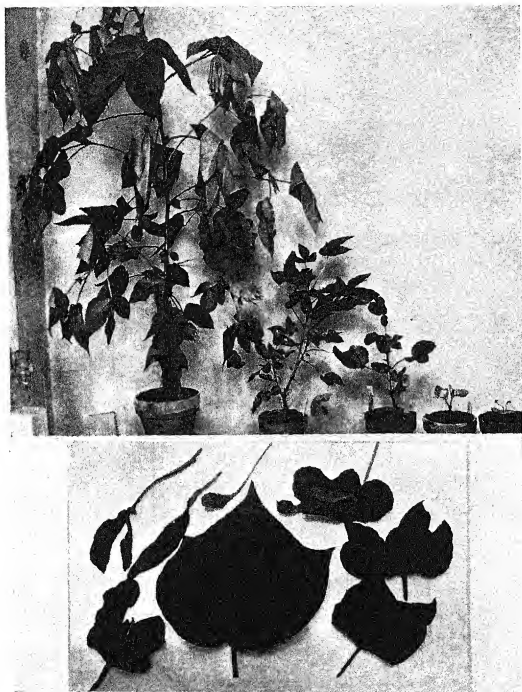
flats or pots in good soil and propagated in a greenhouse. Some of them were later transferred to outdoor conditions, the remainder being retained under glass.

The plants illustrated represent certain of the more striking variations obtained, indicative of the type of physiological modifications most commonly met with.

COTTON.—The cotton seeds used were of a strain of full Sea Island, kindly supplied by Dr. A. F. CAMP of the University of Florida, and exposed as described for 0.5, 2, 4, 8, 16, 32, and 64 minutes. A marked change in growth rate was evident early (fig. 1), none of the plants exposed for 32 minutes attaining more than one pair of true leaves, while extreme fasciation was evident in a large percentage of the 16-minute group. In many cases development in the last-named group proceeded from a lateral bud, the terminal bud failing to develop or doing so only to a very limited degree. Leaf form was markedly altered in a considerable percentage of the higher exposures, the changes being reflections, for the most part, of tissue duplication, inversion, and replacement (fig. 2). Complete dwarfing was not uncommon, the entire plant, including leaves, stems, and flower buds being affected so that symmetry was maintained. In such case, however, it was usual for flower development to be inhibited to the extent that the fully developed, perfect but miniature buds failed to open, passed through the typical color change from yellow to red characteristic of Sea Island, and withered while still closed. Fertilization was accomplished, however, and miniature but perfect bolls were set.

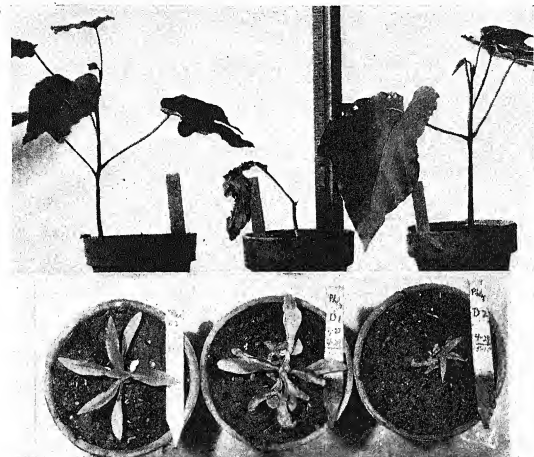
TUNG OIL.—Particular interest attached to the treatment of seeds of *Aleurites* because of their unusual bulk, and because little work has been done in this field. The changes produced with hard x-rays were of the same type as those obtaining with cotton. Fasciation, change of growth rate, chlorophyll defects, and tissue duplication, inversion, and replacement with corresponding alteration of leaf-form, were all in evidence (fig. 3). Further work with tung oil is planned.

SNAPDRAGON, PHLOX.—The modifications figured for *Phlox* (fig. 4) and *Antirrhinum* (fig. 5) were typical for a considerable proportion of the population of these plants. The first is of interest as rep-



FIGS. 1, 2.—Fig. 1 (above), Sea Island cotton at bearing age (4 months) showing progressive dwarfing, fasciation, and leaf deformity: left,—control; left center,—treated dry for 32 minutes (main development made from a lateral bud); center,—treated dry for 16 minutes; right center,—treated dry for 64 minutes (complete inhibition of development in true leaves); right,—treated dry for 64 minutes (tissue deformities in first true leaves; second pair never developed). Fig. 2 (below), leaf deformations and duplications in Sea Island cotton; control at center.

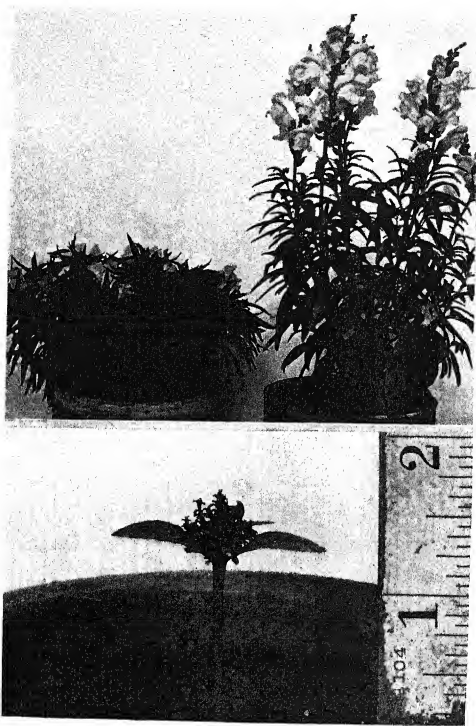
resenting an unusual case of extreme fasciation in which flower color and form has remained practically unaffected, the change being confined entirely to neighboring tissue of leaf and stalk. No further physiological modifications were noticed for *Antirrhinum* in a very



FIGS. 3, 4.—Fig. 3 (above), seedlings of *Aleurites* from x-rayed seeds at age of 3 months: left,—treated dry for 8 minutes (duplication of apical portions of leaf); center,—treated dry for 8 minutes (dwarfing and extensive leaf deformity, the latter a result of tissue replacement and inversion for the most part); right,—treated wet, 0.5 minute (equivalent to control). Fig. 4 (below), *Phlox drummondii* var. *grandiflorum*, from x-rayed seeds at age of 1.5 months: left,—treated dry for 2 minutes (equivalent to control in external appearance); center,—treated dry for 1 minute (extensive and abnormal development of lateral buds); right,—treated dry for 2 minutes (dwarfing and leaf deformation and duplication).

considerable population, but it is to be noted that the limit of tolerance of the dry seed had not been approached closely.

Alterations in growth rate, leaf form, leaf pigmentation, and plant habitus similar to those illustrated were not infrequent in a popula-



FIGS. 5, 6.—Fig. 5 (above), *Antirrhinum* from x-rayed seeds at age of 3.5 months: left,—4-minute exposure (equivalent to control); right,—2-minute exposure (dwarfing and fasciation of stems and leaves; flowers normal in size, coloration, and method of attachment). Fig. 6 (below), *Citrus aurantium* from seed x-rayed for 128 minutes at age of 7 months (extreme fasciation and inhibition of leaf development; at this age the controls of this normally rapid-growing species measured nearly 10 inches).

tion of several hundred specimens of *Phlox drummondii*, and duplication of leaf tissue was noticeable, but no alteration of flower color or form which could be attributed to x-rays was noticed.

SOUR ORANGE.—Seeds of *Citrus aurantium* were given very long exposures to the high-voltage rays in the course of a program to determine the limit of tolerance for the species. A very considerable number of aberrations were obtained among the resulting plants, most of which will require further investigation before they can be reported on. Figure 6, however, represents a rather striking and clear case of extreme fasciation without leaf deformation, since the leaves, although diminutive, are of normal form. Clearly the primordia only of the later true leaves were affected, for the plant appeared normal in the first few weeks of life. Control specimens from the same stock, propagated under similar conditions, grew vigorously, as is usual with this orange, and had attained a height of 10 inches at the time that the figured plant was photographed.

RESEARCH LABORATORY
GENERAL ELECTRIC COMPANY
SCHENECTADY, NEW YORK

PLEISTOCENE FORESTS OF CENTRAL ILLINOIS
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 444

JOHN VOSS
(WITH ONE FIGURE)

The character of the plant life which prevailed during the glacial and interglacial stages is a field of promising interest, which as yet is scarcely developed. Carbonaceous soil belonging to the Sangamon interglacial and Wisconsin glacial stages is to be found in some counties of central Illinois, being sometimes exposed in stream or road cuts, or encountered in well drillings and test borings for coal. The relationship of the Sangamon and Wisconsin to other Pleistocene stages is given by LEIGHTON (4) as follows:

STAGE	SUBSTAGE
	Late Wisconsin
Fourth glacial.....Wisconsin	Early and Middle Wisconsin
	Iowan.....Peorian loess
Third interglacial.....Sangamon	
Third glacial.....Illinoian	
Second interglacial.....Yarmouth	
Second glacial.....Kansan	
First interglacial.....Aftonian	
First glacial.....Nebraskan	

Following the recession of the Illinoian ice sheet, the Illinoian till during the early part of the Sangamon epoch was weathered for a long period of time, resulting in the development of a fully mature soil profile. Toward the close of the period, the Illinoian till was covered with a loess deposit, the upper surface of which also shows a definite soil profile with leaching several feet deep. The strongly developed soil profile is ample evidence that a long period intervened between the deposition of the late Sangamon loess and that of the overlying Peorian loess. According to LEIGHTON, the Peorian loess was laid down partly while the Iowan ice was retreating and partly while the Early Wisconsin ice was advancing. In all the Pleistocene

sections here mentioned the Peorian loess is covered with thick deposits of Early Wisconsin loess.

During the Late Sangamon stage and the period of Peorian loess deposition, shallow undrained depressions were formed on the upland plains. Because of a certain degree of imperviousness of the soil and the probably cool temperate conditions, with abundant regularly distributed rainfall and high humidity, peat accumulated in these basins. This peat varies in color and texture, and often contains well preserved trunks of trees lying in a horizontal position and flattened by the pressure of the overlying material.

SAVAGE (5) and BAKER (1) base their conclusions regarding the type of climate which existed during the Pleistocene largely on the character of the macroscopic plant and animal remains found in peat and loess deposits. Since not all woods have the same resistance to decay and therefore would not all be preserved in peat, the writer undertook the study of the plant remains in this peat by employing the method of pollen analysis, now being used in tracing the succession of vegetation in postglacial peat.

Pollen grains of some plants are preserved in peat long after the plant which produced them has decayed, while other pollen grains soon decay and leave no recognizable trace. The latter statement, according to ERDTMAN (2) and SEARS (6), is especially true of *Larix* and *Juniperus*. ERDTMAN (3) states that the preservation of pollen is often governed by the condition of the bog into which it falls. If the condition is such that the pollen grains remain on the surface of the bog, resulting in exposure to the oxygen of the atmosphere and to continuous changes in humidity, this will prevent their preservation in a fossil state. If they sink to deeper levels, however, where the oxygen supply is limited, it is likely that they will be preserved. In general, pollen grains are not so abundant in the interglacial peat bogs as they are in the postglacial ones. No doubt many pollens have disintegrated since the accumulation of the Late Sangamon peat, for it is estimated that at least 60,000 years have elapsed since the time of its formation. Pollen grains are often compressed through the weight of the overlying soil, a factor which makes it difficult at times to distinguish one genus of conifers from another.

Samples were collected from the following widely separated exposures in Illinois:

1. A ravine of Richland Creek in Section 20, Town 28 North, Range 2 West, Woodford County.
2. The west bank of East Bureau Creek in Section 8, Town 16 North, Range 10 East, Bureau County.
3. A ravine of Farm Creek in Section 29, Town 26 North, Range 3 West, Tazewell County.

Of the three exposures, the last one is by far the most productive, and is probably the most notable one in the United States. In the Farm Creek cut, the buried peat is exposed for a distance of 150 feet and is 11 feet thick. The bottom of the peat stratum is 30 feet above the low-water level of the stream and the top is covered with approximately 60 feet of Early Wisconsin soil (fig. 1).

The samples were obtained by digging back 2 feet or more from the face of the exposure, in order to obtain soil which had not recently been exposed to the atmosphere. Samples were taken every inch near the top and bottom of the peat and every 6 inches in the remainder of the layer. Whenever fragments of wood were found, they were collected and later identified in the laboratory.

At first great difficulty was experienced in separating the pollen grains from the silty matter. The best results were obtained by treating the peat with 20 per cent nitric acid for at least 2 hours. The solution was then centrifuged; a small quantity was removed by means of a pipette from near the surface of the solid material at the bottom of the centrifuge tube, and placed on a microscope slide. The number of pollen grains per square centimeter (in other words, the pollen frequency of the interglacial peat) was exceptionally low as compared with the general pollen frequency of postglacial material. For that reason many slides had to be examined before at least fifty pollen grains per sample were found. Tables I-III show the pollen occurrence in the interglacial peat deposits.

The pollen analyses of the peat given in the tables show that the conifers *Abies*, *Picea*, and *Pinus* were the dominant trees during the Late Sangamon and Early Peorian stages. Since pollen grains of *Picea* outnumber the others, they are probably from the common

bog spruce, *P. mariana*. All the wood collected from the three exposures proved to be *Larix* sp.

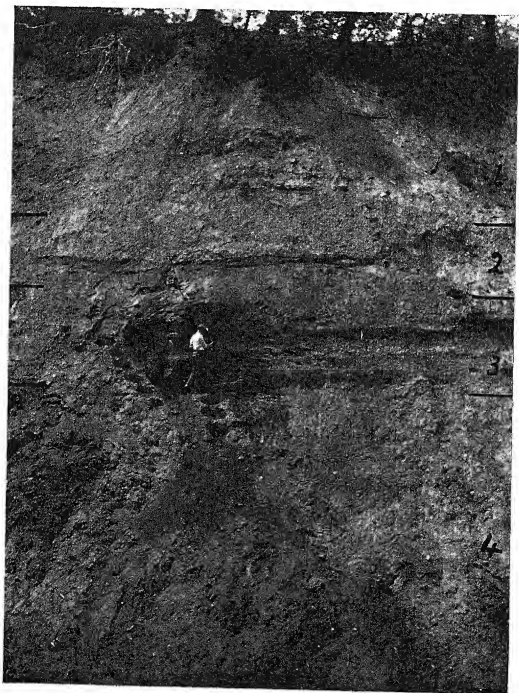


FIG. 1.—Peat exposure at Farm Creek, Tazewell County, Illinois: 1, Early Wisconsin loess, gravel and till; 2, Peorian loess, unweathered; 3, Late Sangamon peat; 4, Illinoian till, with weathered horizon.

TABLE I
POLLEN OCCURRENCE IN RICHLAND CREEK EXPOSURE

DEPTH IN INCHES	CHARACTER OF DEPOSIT	POLLEN			
		ABIES	PICEA	PINUS	POLLEN FREQUENCY
EARLY PEORIAN					
1.....	Yellow clay	0	0	0	0
2.....	" "	0	0	0	0
3.....	" "	0	0	0	0
4.....	" "	0	0	0	0
5.....	Dark gray, woody, Picea leaves	7	38	12	1/2
6.....	" " " "	12	36	9	1
12.....	Black, woody, Picea leaves	6	41	14	2
18.....	Yellow clay	0	0	0	0
24.....	" "	0	0	0	0
30.....	Brown, woody, Potamogeton	7	42	4	1
36.....	" " " "	4	44	6	1/2
42.....	Brown, woody, Picea leaves	6	41	6	1/2
48.....	Brown, few Potamogeton	3	44	2	1
54.....	Brown, many Potamogeton	5	42	5	1
60.....	Brown, woody	9	36	6	1
61.....	Blue clay	0	0	0	0
62.....	" "	0	0	0	0
63.....	" "	0	0	0	0
64.....	" "	0	0	0	0

TABLE II
POLLEN OCCURRENCE IN EAST BUREAU CREEK EXPOSURE

DEPTH IN INCHES	CHARACTER OF DEPOSIT	POLLEN			
		ABIES	PICEA	PINUS	POLLEN FREQUENCY
EARLY PEORIAN					
1.....	Yellow clay	0	0	0	0
2.....	" "	0	0	0	0
3.....	" "	0	0	0	0
4.....	" "	0	0	0	0
5.....	Brown	11	38	9	1
6.....	"	9	43	6	1
12.....	Gray, slightly woody	4	48	4	2
18.....	Gray, woody with Picea leaves	7	45	8	2
24.....	Black, very woody, Potamogeton	9	41	5	3
30.....	" " " "	7	39	8	1
36.....	Black, very woody, Picea leaves	12	42	4	1/2
42.....	Dark red, very woody	8	43	5	1/2
48.....	Dark red, not woody	0	0	0	0
52.....	Brown	0	0	0	0
53.....	"	0	0	0	0
54.....	"	0	0	0	0

In the Farm Creek exposure the top of the Early Peorian is overlain with 5 feet of gray, fossiliferous Late Peorian loess. A thin

TABLE III
POLLEN OCCURRENCE IN FARM CREEK EXPOSURE

DEPTH IN INCHES	CHARACTER OF DEPOSIT	POLLEN			
		ABIES	PICEA	PINUS	POLLEN FRE- QUENCY
EARLY PEORIAN					
1.....	Yellow clay	0	0	0	0
2.....	" "	0	0	0	0
3.....	" "	0	0	0	0
4.....	" "	0	0	0	0
5.....	Yellow clay with small pieces of wood	11	37	4	1
6.....	Dark gray, Picea leaves, Potamogeton, small twigs	9	40	6	2
12.....	Dark gray, Picea leaves, Potamogeton	13	32	8	2
18.....	" " " " "	8	39	2	1/2
24.....	" " " " "	7	47	5	1/2
30.....	" " " " "	0	0	0	0
36.....	Dark gray, wood 3-4" diameter	0	0	0	0
42.....	Light gray, very woody, many Picea leaves	13	42	6	1
48.....	Black, very woody	0	0	0	0
LATE SANGAMON					
54.....	Black, very woody	9	45	1	2
60.....	Black, logs 4" diameter, Picea leaves	8	41	2	1/2
66.....	" " " " "	10	47	9	1/2
72.....	Black, very woody	0	0	0	0
78.....	Gray, no wood	0	0	0	0
84.....	Light gray	0	0	0	0
90.....	Black, woody, Picea leaves	5	48	13	5
96.....	Black, no wood	7	46	15	3
102.....	Dark brown, no wood	12	41	8	7
108.....	Dark brown, few Picea leaves, Pota- mogeton	11	39	11	3
114.....	Black, extremely woody, Potamogeton	10	42	8	4
120.....	Black, no wood	13	47	14	5
126.....	Yellow	0	0	0	0
132.....	Dark brown	8	41	5	1/4
133.....	" "	0	0	0	0
134.....	" "	0	0	0	0
135.....	" "	0	0	0	0

humus layer was found at the top of the Late Peorian in which the following mosses were found: *Hypnum curvifolium* (Hedw.), *Calliergon giganteum* (Schimp.) Kindb., *Calliergon* sp., and two species of

Plagiothecium. Branches and roots of *Larix* sp. were found scattered throughout the Late Peorian loess, and were especially abundant just below the humus horizon.

Summary

The data seem to warrant the conclusion that forests of *Abies*, *Picea*, *Pinus*, and *Larix* existed in central Illinois for many centuries during the Late Sangamon and Early Peorian periods. Macroscopic remains also show the presence of *Larix* during the Late Peorian stage. The climate was apparently the same as that of the region in which the northern conifers are now found. This area extends in the north from Labrador and Newfoundland to northern British Columbia and the Yukon, and in the south includes the northern parts of Michigan, Wisconsin, and Minnesota.

Grateful acknowledgment is here made of my indebtedness, for valuable suggestions and advice, to Dr. GEORGE D. FULLER, of the University of Chicago, and to Dr. M. M. LEIGHTON, Chief of the Illinois State Geological Survey. To the Forest Products Laboratory, Madison, Wisconsin, to Dr. SAMUEL J. RECORD, of Yale University, to Dr. A. P. DACHNOWSKI-STOKES, of the U.S. Department of Agriculture, and to Dr. SEVILLE FLOWERS, I am indebted for the identification of the macroscopic plant remains.

MANUAL TRAINING HIGH SCHOOL
PEORIA, ILL.

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ABNORMALITY IN SWEET CHERRY BLOSSOMS AND FRUIT

G. L. PHILP

(WITH FIVE FIGURES)

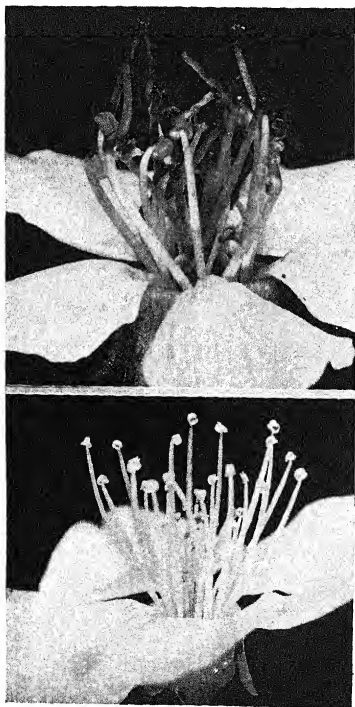
Many abnormal sweet cherry (*Prunus avium*) flowers were observed during the spring of 1932. In the midseason varieties, Napoleon, Bing, and Hoskins, many flowers showed several pistils extending through the tip of the unopened blossom. Upon examination it was discovered that these protruding pistils instead of anthers occurred at the tips of the filaments (fig. 1). Figure 2 shows a normal blossom. In many cases abnormal development of the petals had also occurred (fig. 3). An occasional abnormal blossom had been observed in other years, but in 1932 so many were encountered, particularly in the Napoleon and Bing varieties, at the University Farm, Davis, California, that it was decided to investigate the extent of this abnormality.

There are extensive cherry plantings in Santa Clara, Sonoma, Napa, Solano, Sutter, Placer, and San Joaquin counties. Observations were made in all of these areas for abnormal cherry flowers, and at harvest season fruit was examined. It was found that the occurrence of abnormal flowers was extensive in the hot interior valleys of the Sacramento and San Joaquin, becoming considerably less in the Sierra Nevada foothills to the east and also in the foothills to the west. No abnormal flowers were found in the coastal valleys of Santa Clara, Napa, and Sonoma, where summer temperatures are lower.

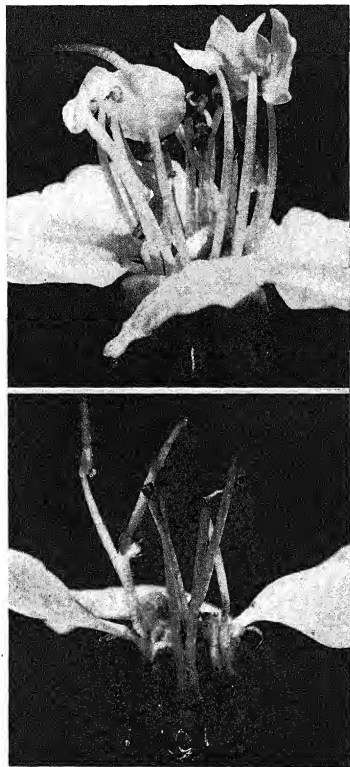
Practically all the abnormal flowers contained double pistils (fig. 4), which upon development produced double or malformed fruits (fig. 5). Fruit counts, however, indicated that many otherwise normal blossoms contained double pistils as illustrated in figure 4.

In a cherry orchard in Sutter County, a number of spurs having abnormal blossoms were tagged. All the normal flowers were removed and only flowers showing abnormal pistil or petal development were counted. No record was made as to whether or not the ab-

normal flowers contained double pistils. Table I gives the results of these counts.



FIGS. 1, 2.—Fig. 1 (above), abnormal cherry flower; note abnormal pistils attached to end of filaments. Fig. 2 (below), normal cherry blossom; *cf.* fig. 1.



FIGS. 3, 4.—Fig. 3 (above), abnormal petal development on end of filaments. Fig. 4 (below), in addition to abnormal pistil development on top of filaments, note the double pistil.

On one limb of a Bing tree 126 fruits matured, 74 of which were normal, 18 double, and 34 malformed. In 200 Napoleon fruits from this orchard (orchard run), 92 were normal, 50 were doubles, and 58 were malformed. There was a greater percentage of abnormal fruits near the ends of the branches. At harvest, counts were made of Napoleon cherries from a 10-year-old tree at the University Farm, segregated as terminal fruit (less than 3 feet from the ends of the

TABLE I
FRUIT DEVELOPMENT FROM ABNORMAL CHERRY BLOSSOMS

VARIETY	NO. OF ABNORMAL BLOSSOMS	FRUIT MATURED		
		NORMAL	DOUBLES*	MALFORMED†
Bing.....	119	0	5	11
Napoleon.....	234	1	1	18

* Double fruit: both ovaries developed equally.

† Malformed fruit: one ovary completely developed while other is only partially so (fig. 5).

branches) and inside fruit (more than 3 feet from the ends of the branches). The results are given in table II, which also gives the analysis of a composite sample of terminal and inside fruit, collected from six trees in the orchard selected at random. Table II shows that a greater percentage of doubled and malformed fruits occurs near the ends of branches than farther back on the limbs.

Table III segregates the number of normal, double, and malformed fruits from the entire crop from a Napoleon tree at the University Farm. The development of doubles and malformed fruits was not confined to the sweet cherry. While it was most prevalent with Napoleon and Bing varieties, it was also common in Montmorency (*Prunus cerasus*). The results of a composite sample from ten Montmorency trees are shown in table III.

It was generally observed that the Napoleon and Bing varieties produce many double and malformed fruits in the interior valley and none or very few in the coastal regions. It was also found that the percentage of double and malformed fruit varies considerably from year to year. Very few abnormal fruits occurred in 1931, in the in-

terior valleys, while the tables show a significant number in 1932. Temperature records at Davis show a total of 20 days in 1930 when the temperature reached 100° F. or above. In 1931 there were 30

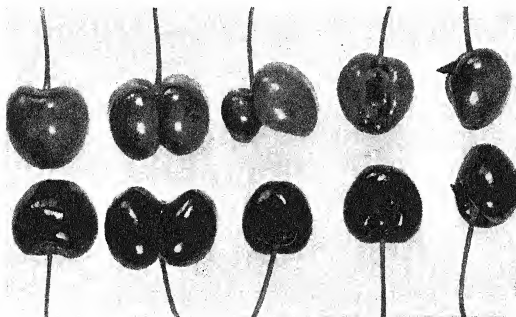


FIG. 5.—Top row, Napoleon; bottom row, Bing: normal fruit at left, from development of normal flower; other fruits showing varying degrees of development of double pistils.

TABLE II

NAPOLEON CHERRIES	NUMBER OF FRUITS					
	NORMAL		DOUBLES		MALFORMED	
	Terminal	Inside	Terminal	Inside	Terminal	Inside
From single 10-year-old tree	157	276	63	26	195	133
From six trees (composite sample).....	275	377	74	46	284	169

days with a temperature of 100° F. or above; also 20 of the 30 hot days occurred during July, while only 7 days in 1930 for July were above 100° F.

Records in the Santa Clara Valley show no temperature of 100° F. for 1930 and only four days in 1931 when the thermometer reached

or exceeded this temperature. The Davis records show that the mean maximum temperatures for July and August, 1931, were 101.3 and 97.2 respectively, while for 1930 they were 94.2 and 94.4, indicating a much warmer summer for 1931 which was followed in 1932 by a significant number of abnormal blossoms and fruits.

TABLE III

SAMPLE	NUMBER OF FRUITS		
	NORMAL	DOUBLES	MALFORMED
Entire crop from a 10-year-old Napoleon tree.	1013	216	1797
Composite sample of a Montmorency crop.	557	85	243

Calculations by TUFTS¹ show the total heat units for July, 1931, to be 1318 while for the same month in 1930 they were 1034, which also indicates much higher temperatures in 1931. TUFTS and MORROW² found fruit-bud differentiation of the Napoleon cherry beginning in early July. It is probable that this abnormal development and the production of double and malformed fruits may be associated with climatic conditions, possibly high temperatures during the summer previous to blossoming, particularly during the period of fruit-bud formation.

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¹ TUFTS, W. P., Unpublished data, University of California. 1932.

² TUFTS, W. P., and MORROW, E. B., Fruit-bud differentiation in deciduous fruits. *Hilgardia* 1:1. 1925.

A SPHENOPTERID FRUCTIFICATION FROM THE PENNSYLVANIAN OF MICHIGAN¹

CHESTER A. ARNOLD

(WITH THREE FIGURES)

In spite of the abundance of fernlike leaf impressions in the Carboniferous rocks, fertile specimens are infrequent, and it is only rarely that specimens are found sufficiently well preserved to show structure.

The fructification constituting the subject of this account was among some fossil plant material collected a few years ago by Dr. W. A. KELLY in a quarry near Corunna. The rock containing the fossils is a very fine grained, dark gray sandstone which effervesces freely when treated with hot acid. The fossiliferous strata exposed at Corunna are considered as belonging to the Saginaw formation of the Pottsville series, which occurs early in the Pennsylvanian system. Associated with the sphenopterid fructification is an abundance of leaves of *Megalopteris* and *Cordailes*, seeds of the *Samaropsis* and *Rhabdocarpus* type, and numerous other Carboniferous plants.

The specimen under consideration is a small portion of a frond measuring 17 mm. in length (fig. 1). The ultimate pinnules fork three or four times, forming short lobes which depart at acute angles from the main portion of the pinnule. Traversing the center of the pinnule is a simple vein which branches dichotomously, one branch forming the simple vein of the ultimate lobe, the other passing outward to fork again at the base of the next lobe. The lamina along the veins is continuous between the pinnules, is narrow, and varies but little in width. The pinnules are small, narrow, and scarcely more than 5 mm. long.

Since the specimen is but a small portion of a plant with small delicate pinnae, identification is very uncertain. Its affinity with the sphenopterid assemblage of Carboniferous leaf impressions is clearly shown by the narrowly attached pinnules which are wedge-

¹ Contribution from the Museum of Paleontology of the University of Michigan.

shaped (figs. 1, 2). Superficially it bears some resemblance to figures of such forms as *Sphenopteris divaricata* or *S. larischi*, but no attempt is made here to assign it to either of these or to any other species of that genus.

The fructifications are situated at the tips of the lobes of the pinnules (fig. 1). They appear in the impression as small star-shaped structures with the rays attached to a central receptacle. It is difficult to determine just how these structures are related to the pinnule



FIGS. 1, 2.—Fig. 1, fertile portion of frond; fig. 2, sterile portion of frond; $\times 2\frac{1}{2}$

lobes; whether they are attached directly to the laminated lobes or whether they are projected beyond on short filaments. There can be no error, however, in the association of these structures with the pinnules, because each star-shaped fructification is directly beyond the end of a lobe and in a few instances the midvein can be seen extending all the way to the receptacle.

The individual rays are 0.75 mm. long and vary in number from five to eight per group. They appear to be joined, or at least to touch each other for a portion of their length, but with their ends free. Superficially these rays seem individual sporangia attached to a cen-

tral point, as in *Calymmatotheca*; but a closer examination shows that a different interpretation must be applied to them. In a few favorable instances in which the lower surface of these structures can be seen they show as the spread segments of an oval body split several times lengthwise. The presence of spores in some of them indicates their sporangial nature. While living, these sporangia hung downward from a presumably horizontal lamina, and the spread appearance of the rays of the fossil was probably augmented by pressure during fossilization. This position of the unopened sporangia is



FIG. 3.—Spores obtained by maceration; $25 \times 30 \mu$

also indicated by a lateral view of partially opened ones in a few instances. The spores, which appear as small amber colored objects, are approximately $25 \times 30 \mu$ in diameter and appear to have smooth walls (fig. 3).

In its generic affinities, this fructification conforms in essential features to *Zeilleria*. This genus was founded by KIDSTON (3) when he realized that STUR (7) had included two types of fructifications in *Calymmatotheca*. KIDSTON's description of *Zeilleria* is as follows: "Involucres borne at the extremities of the pinnule segments, which are more or less produced to form a pedicel; in the earlier condition the involucres are globular, but at maturity they split into four lobes." In my specimen the number of segments varies from five to eight (fig. 1), but this difference hardly justifies removal from the genus when it is characteristic in so many other ways.

As originally described by STUR, *Calymmatotheca* consists of a globular sporangium which, upon maturity, splits into a number of segments after the manner just described for the *Corunna* specimen. RENAULT (6), however, and later ZEILLER (8) and KIDSTON (4), pointed out that the fructifications originally included in *Calymma-*

lothea are composed of a number of elongated sporangia arranged in a circle around a common point of attachment. Then KIDSTON (3) noted that two species included by STUR in *Calymmatotheca*, *C. avoldensis* and *C. frenzli*, together with *Sphenopteris deliculata*, to quote KIDSTON, "have an indusium which is erstwhile immature, globular, but at maturity splits into four valves." These three species were incorporated into the genus *Zeilleria*. *Calymmatotheca* belongs to the Marattiaceae, KIDSTON believed, while *Zeilleria* belongs to the Hymenophyllaceae. Another difference between the two forms which was pointed out by KIDSTON is that in *Calymmatotheca* the fructifying portions are entirely destitute of foliage leaves, whereas in *Zeilleria* the fertile pinnules differ but little from the sterile forms.

Later, after the discovery of the pteridosperms, KIDSTON concluded that the fructifications of *Zeilleria avoldensis* are seed-bearing cupules, as he claims to have observed slight protuberances where he supposed seeds were attached (5). GOTHAN (2) believes that these cupules are the containers of fernlike spores, and that *Z. avoldensis* is a true fern rather than a pteridosperm. CORSIN (1) has more recently investigated this species and maintains that it belongs to *Pecopteris*, closely resembling *P. miltoni*, which is supposed to be a fern of the marattiaceous type.

From the material at hand it is impossible to form any definite conclusions concerning either the pteridospermous or the filicinean affinities of *Zeilleria*; nor is it yet possible to demonstrate that some species might not belong to one group and some to another. This specimen is different from *Z. avoldensis* but its affinity with the genus is clear. Macerated preparations, however, do show that some of these star-shaped structures are dehiscent spore cases in accordance with GOTHAN's view. Whether these spores are those of ferns or are the microspores of a heterosporous plant (a pteridosperm) would be difficult to determine, since in no instance are both the male and female organs of a given species of a pteridosperm definitely known.

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THE LIQUID WAX OF SEEDS OF SIMMONDSIA CALIFORNICA

ROBERT A. GREENE AND E. OSBORN FOSTER

Simmondsia californica, commonly known in the southwest as "jojobe" or "jojove," is an evergreen shrub 5-15 feet high which is found principally in southern California, Baja California, and in southern Arizona. The seeds are approximately 12 mm. long and 8 mm. thick, and are covered by a thin but hard seed coat. Some time ago, several samples of the beans were submitted to this laboratory for analysis. The average of several determinations showed a content of 45.66 per cent crude fat. The shrub is somewhat hardy and has a low water requirement, so investigations were commenced to determine the nature of the oil which the bean contains and whether there might be some commercial possibilities for it.

Apparently the only study of the beans of this plant has been made by ROEHR (4), who states:

The beans do not contain starch, glucose, tannin, alkaloids, or glucosides. Forty-eight per cent of the seed is soluble in a mixture of chloroform and ethyl ether. This was composed chiefly of fat and an oil of sp. grav. 0.9625 (16° C.). The oil was soluble in the usual fat solvents. The fat (42.87%) melted at 36° C., and had a faint bland odor which resembled oil of theobroma, which was bland to the taste at first, then nauseous. The seeds contain a purging principle which was not isolated, but which might be a toxalbumin similar to ricin.

Approximately 100 pounds of the seeds were received through the courtesy of Mr. F. J. CRIDER, Director of the Boyce Thompson Southwestern Arboretum, Superior, Arizona. The seeds were ground in a power mill, the oil extracted by means of a hydraulic press (300 kg. per sq. cm.), and filtered through a filter on a steam funnel. The yield was approximately 25-30 per cent by weight.

The expressed oil, which was transparent, a golden yellow color, and tasteless, was analyzed, employing the methods of the Association of Official Agricultural Chemists (1). The results were as follows:

Specific gravity 25° C.....	0.8635
Refractive index (Abbé) (25° C.).....	1.4650
Iodine no. (Hanus).....	88.40
Reichert-Meissl no.	0.70
Polenske no.	0.31
Acid value.....	0.23
Soluble acids (as butyric).....	2.43%
Insoluble acids.....	59.43%
Unsaponifiable residue.....	37.62%
Saponification no.	95.00
Acetyl no.	6.80

The results obtained were compared with the characteristics of various oils as given by LEWKOWITSCH (2). It was found that, with the exception of the value for the saponification number, the constants of this oil are practically identical with those of sperm oil and arctic sperm oil, which are liquid waxes.

The oil of *Simmondsia californica* is soluble in ether, petroleum ether, chloroform, carbon tetrachloride, carbon disulphide, and benzene; but is immiscible with 95 per cent alcohol, absolute alcohol, and acetone. The oil solidifies at 10°–12° C. A small amount of it was exposed to the air on a glass plate for several weeks without any evidence of drying.

Early in this study it was realized that this oil had the characteristics of a wax rather than an oil. Consequently a quantity of it was saponified with alcoholic KOH. A homogeneous solution resulted, which separated into two layers when diluted with water. The mixture was acidified in order to liberate the fatty acids, filtered, and the filtrate neutralized with NaOH, concentrated on a steam bath and tested for glycerol. The acrolein test and various color tests were doubtful, indicating that if glycerol is present, it occurs in extremely small amounts.

Another portion of the oil was saponified, diluted with distilled water, and the unsaponifiable material separated by means of a separatory funnel. Qualitative tests, such as employed in organic qualitative analysis, showed the unsaponifiable material to consist chiefly of an alcohol. This alcohol melted at 7° C. and boiled at

231° C. This would indicate that the material might be decyl alcohol. When the oil was subjected to the elaidin test, a solid isomer formed within 15 minutes. Although most waxes usually consist of the fatty acid esters of higher monohydroxy alcohols (cetyl, octodecyl, ceryl alcohol, etc.), RICHTER (3) states that n-hexyl alcohol occurs as acetic and butyric esters in the oil to the seed of *Heracleum giganteum*, and n-octyl alcohol as the acetic acid ester in the volatile oil of the same plant, and as the butyric ester in the oil of *Pastinaca sativa*. Accordingly the presence of decyl alcohol in the seeds of *Simmondsia californica* seems probable.

Sperm oil is a valuable lubricant, and because of the similarity in characteristics, this wax might therefore have some value for lubricating purposes.

Summary

1. The seeds of *Simmondsia californica* contain a liquid wax, which has been tested, and its characteristics and constants agree very closely with those of sperm oil and arctic sperm oil.
2. Qualitative tests indicate that the wax might consist principally of fatty acid esters of decyl alcohol.

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CURRENT LITERATURE

BOOK REVIEWS

Cycads

For many years SCHUSTER has been studying the cycads, especially from the taxonomic standpoint. He has now given a helpful account¹ of the present status of the classification, geographic distribution, and paleontological development of the group.

The known fossils are described critically and some are allowed a place in the cycadophyte phylum; some are thought to be ferns, and it is suggested that some are even palms or other angiosperms. In the case of some which are given a place in the cycadophytes, the author believes the evidence is not sufficient to determine whether the forms should be assigned to the Bennettiales or the Cycadales. Little importance is given to leaf characters in distinguishing between these two orders. The Mesozoic *Pterophyllum*, *Dioonites*, *Otozamites*, *Psilophyllum*, and *Podozamites* appear in the Triassic (Rhaetic), only *Pterophyllum* going back to the Carboniferous, and they became extinct in the middle Cretaceous. *Zamites*, *Anamozamites*, *Glossozamites*, *Nilssonia*, and *Cycadites* became extinct in the middle Jurassic. A large part of these forms doubtless belonged to the Bennettiales, which extend from the Keuper to the upper Jurassic; but megasporophylls (*Cycadospadix* in the Rhaetic and *Zamioidea* in the Keuper) show that the Cycadaceae were also present. The Bennettiales and Cycadales are parallel developments from the Paleozoic Cycadofilicales.

In geographic distribution the Mesozoic cycadophytes were cosmopolitan, from Greenland, Alaska, and Siberia to Patagonia, New Zealand, and South Africa and all the way around, a striking contrast to the present condition, with the Bennettiales entirely extinct and the Cycadales confined to tropical and subtropical regions.

SCHUSTER thinks that the origin of the recent genera cannot be determined by the paleontological evidence, but must be conjectured from geographical and geological evidence. The reviewer would agree that little dependence can be placed upon leaves as a criterion for distinguishing between Bennettiales and Cycadales; but, for that very reason, many of the leaves assigned to Bennettiales may belong to Cycadales. Besides, if the fructifications of the ancient Cycadales decayed early, like those of the living forms, preservation would be

¹ SCHUSTER, JULIUS, Über das Verhältnis der systematischen Gliederung, der geographischen Verbreitung und der paläontologischen Entwicklung der Cycadaceen. Ein Beitrag zur historischen Biologie. ENGLER'S Botanische Jahrbücher 64: 165-260. pls. 4-11. 1931.

rarer than in the better protected fructifications of the Bennettiales. An occasional sporophyll, like *Cycadospadix hennoquei*, is well preserved, and it seems to differ less from sporophylls of the living *Cycas revoluta* than sporophylls within this genus differ from each other. The rapid decay of fructifications and the assigning of leaves to Bennettiales may account, in some degree, for the incompleteness of the fossil record of Cycadales.

SCHUSTER says that when we speak of a cosmopolitan distribution of the cycadophyte phylum we refer to the Bennettiales, not to the Cycadales, since the cycads in the beginning, as now, were scantily represented. Their ancestors were transitional forms between the Cycadofilicales and the modern Cycadales, arising in the upper Triassic as climatic reactions to the aridity of the Eurasian continent. These ancient forms gave off a branch to Gondwanaland and one to North America. From the descendants of these the present-day cycads arose from genetic centers in Himalaya, West Australia, South Africa, Mexico, and Central America.

The earliest trace of the Cycadaceae is found in the upper Triassic (Keuper), with *Cycadospadix*, the forerunner of *Cycas* and *Beania*, of *Zamia*, both Eurasian. Only one species of the living cycads has been found as a fossil, a species of *Cycas* in the older Tertiary of Japan. In the upper Triassic, a branch of the Zamiodeae reached North America. The Permian *Autunia* and *Cycadoxylon* show that the family has come from the Cycadofilicales.

The living genera of cycads are parallel developments, none having been derived from any other living forms. *Cycas* and the American genera had a north continental origin; while the African and Australian genera, with the exception of *Cycas*, were southern. Neither the Bennettiales nor the Cycadales gave rise to the angiosperms.

The paper contains an immense amount of information, critically organized, and references to literature are adequate; but such an extensive bibliography would have been more conveniently arranged chronologically or alphabetically at the end of the contribution.—C. J. CHAMBERLAIN.

Nature's gardens and man's gardens

A number of recent books serve to emphasize the social nature of plants, whether in natural communities or in the gardens planned and planted by man. A volume of this type by WELLS describes in simple terms the plants and plant communities of North Carolina.² The material is organized under two general headings, The natural gardens of North Carolina, and The herbaceous wild flowers of the natural gardens. The first part consists of descriptions of the principal plant communities of the state, the second of descriptions of the chief individual plant species.

The ecological descriptions are in non-technical language that may at times be criticized as teleological. The facts presented in the discussion, however,

² WELLS, B. W., The natural gardens of North Carolina. pp. 458. figs. 209. University of North Carolina Press. Chapel Hill, N. C. 1932. \$3.50.

seem to be well selected, accurately stated, and calculated to enable the lay reader to enjoy the landscapes of grassland, forest, and mountain with a more intelligent interest. This interest will be increased by the 123 illustrations in the first part of the book, which covers 208 pages.

In the second section are found keys and descriptions of individual plant species. The seven keys are for the plants of seven great community types. They are for the herbaceous plants of: great forest uplands, sand hills, coastal dunes, savannas, fresh-water marshes, salt marshes, and aquatic plants. In the keys, flower color and leaf form play an important part, and the general scheme seems a promising one. The descriptions of individual species are brief and simple, but aided by the numerous illustrations, will be found useful.

The year has also had an abundance of books about plants that are grown in gardens. This is particularly true of a group from the house of Macmillan. The first is a book describing the plants of alpine communities that provide the materials for the rock gardens of the lowlands.³ The author deals with the mountains of the west, including Rainier, Hood, and the neighboring peaks and ranges. Perhaps the best part of the book is the remarkable collection of over 100 photographs of alpine plants and flowers from these mountains. There are, however, descriptions of the plants and directions for growing them in rock gardens.

A third volume is by one⁴ who had two extensive Florida gardens, which were really small arboreums, including all the tropical species found in the state, together with many from other lands. He knew these plants intimately; their names, classification, requirements, habits, and beauty, and wrote about them from his rich store of information. His descriptions are sympathetic but also scientifically accurate. The gardener has now passed away, but his writings have been collected and put into a book of fascinating interest by two of his friends.

The next in the series of garden books⁵ has a very different scope. It is by an English writer, who has visited the natural and cultivated gardens of America, from Vancouver to California, Arizona, Louisiana, South Carolina, Maryland, and Maine. It is the story of a garden pilgrimage charmingly told, in spite of the occasional misuse of scientific names.

In the first part of another book⁶ the reader may find a vast amount of plant

³ GABRIELSON, IRA N., *Western American alpine*. pp. xviii+271. figs. 121. Macmillan Co. New York. 1932. \$3.50.

⁴ NEHRING, HENRY, *The plant world of Florida*. From published manuscripts collected and edited by ALFRED and ELIZABETH KAY. pp. xiii+304. pls. 27. Macmillan Co. New York. 1933. \$3.50.

⁵ CRAN, MARION, *Gardens in America*. pp. 320. pls. 16. Macmillan Co. New York. 1932. \$3.50.

⁶ FOX, HELEN M., *Gardening with herbs for flavor and fragrance*. pp. xiv+334. pls. 12. Macmillan Co. New York. 1933. \$3.50.

lore, together with much historical information regarding plants used for their flavors or their odors. As most aromatic plants have been regarded as possessing medicinal properties, the old "herbals" and the "doctrine of signatures" abound in curious statements about the mythical qualities of herbs. The author has drawn much from these sources. The herb lore of the United States is shown to be a mixture of that imported from Europe and that found among the North American Indians.

In the second part of the book the author describes some 70 herbs, giving for each the scientific name, a short description, its history and legend, its uses, and its culture. Several of the plants are illustrated by drawings that are artistically pleasing but would be of little assistance in the recognition of the plants. There are ten pages of bibliography.

Similar to the last, but covering a much wider field, WILDER⁷ has drawn from personal experience, from catalogues, from old herbals, and from a variety of other sources a most extensive list of fragrant plants. These are described as to their botanical classification, their place in gardens, and their native homes. They are segregated into groups according to their odors and discussed on this basis.

This book too has its bibliography, but the author has drawn from poetry and history as well as from textbooks and scientific journals. An extensive index of both common and scientific names makes its information easy of access. It will make a valuable addition to our shelf of garden books.—G. D. FULLER.

Handbook of plant analysis

Earlier volumes of the handbook of plant analysis by KLEIN⁸ have been noted in this journal.⁹ The third volume comes in two large tomes with consecutive paging. The first half contains 806 pages, and the second 807.

The first half of volume III considers a few large groups of substances: membrane substances; naturally occurring tannins; lichen substances (acids, fats, etc.); ethereal oils; caoutchouc and gutta-percha; and resins. The second half is divided into a greater number of sections, but the substances considered really fall into two large groups, glucosides and plant pigments. Among the glucosides the following are included: glucosides with aliphatic and aromatic substances; flavones and related compounds; anthocyanins; anthracene glucosides; hydrocyanic acid glucosides; indoxyl glucosides; garlic and mustard oils and their glucosides; saponins; digitalis glucosides; and glucosides of little understood composition. The pigments considered are the carotinoids, chlorophyll, algal pigments, bacterial and fungal pigments, and other pigments which have

⁷ WILDER, LOUISE B., *The fragrant path*. pp. xv+407. Colored frontispiece. Macmillan Co. New York. 1932. \$3.00.

⁸ KLEIN, G., *Handbuch der Pflanzenanalyse*. III/1, 8vo. 1-806; III/2, 8vo. 807-1613. J. Springer. Vienna. 1932.

⁹ BOT. GAZ. 92:332-333. 1931; *Ibid.* 94:427-428. 1932.

not been thoroughly investigated, such as rottlerin, santalin, vitexin, gossipol, trifolitin, etc.

The methods of detection, quantitative estimation, and preparation methods are usually given, and the occurrence and systematic distribution of the various substances in the plant kingdom are given for most of the substances included. The vast amount of information contained in these volumes is indicated by the fact that the index alone occupies 137 pages.

Generous space is given to the consideration of each group of substances. Thus in the first part of the volume, the membrane substances, cellulose, hemicelluloses, gums, mucilages, chitin, pectins, lignin, cork, cuticle, crude fiber, and phytomelane; and the membranes of algae, bacteria, fungi, mosses, and ferns; and fossil plant membranes, occupy 344 pages. Numerous literature lists are provided, with citations of recent literature. Brief sketches of the background and development of our knowledge give the work the illuminating historical perspective so much needed in current research.

The editor and publishers have provided an indispensable handbook for the use of plant chemists, plant physiologists, and all investigators whose work makes necessary a study of the products of plant metabolism.—C. A. SHULL.

Root nodule bacteria

It is fortunate for botanists that FRED²⁰ and his associates have summarized their work and given it setting in a monographic treatise on the root nodule bacteria and leguminous plants. This monograph contains 13 chapters devoted to: the history of Leguminosae in agriculture; general description of the Leguminosae; their distribution and importance; the occurrence of root nodules; isolation and study of root nodule bacteria; their morphology and life cycle; their cultural and biochemical characteristics; factors which influence growth and longevity of nodule bacteria; their species relationships; formation of nodules, their histology and cytology; relationship between leguminous plants and bacteria; factors influencing nodule production; economic importance of leguminous crops; natural and artificial inoculation. An appendix deals with practical problems such as use, distribution, inspection, and testing of artificial cultures. The bibliography consists of almost 60 pages of citations. The plates are excellent; there is a good index; and also a frontispiece with a portrait of HERMAN HELLRIGEL, and a dedication to the late WILLIAM HARMON WRIGHT.

The subject matter of this field has been of continuous practical interest to man since his first attempts at agriculture. It is full of meaning for the microbiologist, the ecologist, the pathologist, the physiologist, the morphologist, the histologist, and the cytologist. It has many theoretical implications, especially for those interested in the fundamental problems of infection, pathogenesis, aggressivity and virulence, susceptibility and resistance; cellular, tissue, and organ re-

²⁰ FRED, E. B., BALDWIN, I. L., and MCCOY, E., *Root nodule bacteria and leguminous plants*. Univ. of Wisconsin Studies in Science, no. 5. pp. xxii+342. pls. 46. Madison, Wisconsin. 1932.

sponses, as well as responses of the plant as a whole to non-pathogenic and pathogenic infection. The reader will find data bearing on all of these points. He will find that while much is known, much more still remains to be discovered. It is one of the merits of this treatise that while it ably and clearly presents the known, it also deliberately calls attention to the unknown. It is obvious that ascent of the next range of peaks involves many difficulties and much preliminary trail-making of a biochemical and cytological nature.—G. K. K. LINK.

Methods

The fifth edition of the text on methods by CHAMBERLAIN¹¹ has been almost entirely rewritten, with new illustrations. Many things are presented for the first time. Some obsolete methods are omitted; others are retained because of historical interest.

In part I are chapters devoted to apparatus, reagents, stains and staining, a concise history of staining (including theories and practical hints on staining), temporary mounts and microchemical tests, freehand sections. The glycerin method, although practically obsolete, is described because there are some forms which still must be mounted in media with a glycerin base. The Venetian turpentine method is presented with concise and workable directions. The paraffin method is given the thorough treatment commensurate with its importance in modern technique. The now almost obsolete celloidin method is described because of its historical interest. The chapter on special methods gives those worked out by the author as well as those developed by research workers in very special fields. The chapter on paleobotanical technique gives the latest methods in use. The chapter on botanical photography is the result of the author's wide experience in this subject, extending over many years. The portion of this chapter written by PAUL J. SEDGWICK, entitled *Movie Photomicrographs*, should enable any one familiar with ordinary photographic methods to make successfully motion picture photomicrographs. The new chapter describing how to prepare illustrations for publication is most timely.

In part II the general principles given in part I are made specific for all the great groups of plants, since the author realizes the difficulty which beginners especially have in applying general principles to concrete cases. This part is illustrated by excellent illustrations which should serve as models for future illustrations. It is a far better textbook of botany than most of the current so-called textbooks.

The book is the result of more than 40 years of intensive study and first-hand contact with botany; and in the opinion of the reviewer, is the last word on modern botanical technique.—W. J. G. LAND.

Plants of the Rocky Mountain National Park

During the past decade the National Park Service has made a decided effort to promote the study of the vegetation of the various parks within its system.

¹¹ CHAMBERLAIN, C. J., *Methods in plant histology*. 5th ed. pp. 416. University of Chicago Press. 1932.

The Rocky Mountain National Park is one of the best known and most accessible, being visited by a quarter of million persons each year. The vegetation of this park is remarkable for its diversity and for the richness of its alpine flora. A description of its plants by ASHTON¹² cannot but make them better known to the visiting public. With its many illustrations from photographs, botanists may get much assistance upon a first visit to the mountains. The book has an ecological introduction, keys for identification, and descriptions of all the principal species given in simple but accurate language, with both scientific and common names. The latter seem to have been happily chosen so as to lead to as little confusion as possible. Most of the many photographs with which the book is illustrated are of good quality and will assist in the recognition of the plants.—G. D. FULLER.

Plant lore of Palestine

Coming from the pen of one who had spent her life in Palestine and had an intimate acquaintance with its people, this book¹³ brings a strange mixture of Jewish, Moslem, and Christian truth and legend. Most of these legends cluster about useful plants such as cereals, pulse, the vine, and the olive, although medicinal plants, sacred trees, and magical plants are also included. Each plant is designated by its scientific, common English, and Arabic name, the last transliterated from the Arabic alphabet. The use made of the plant by the common people is then given, and all the mystic lore, legend, and poetry that have collected around it are recounted. There seems every reason to suppose that this book presents the true lore of the East, and as such its pages will be a source of much information concerning biblical lands.

The illustrations contain many drawings of the more common plants, affording a good idea of their appearance.—G. D. FULLER.

Potato leaf roll

Although potato leaf roll seems to have been responsible for potato failures in Europe in the last quarter of the 18th century, it was not recognized as a specific disease of the potato until 1905 and was not found in the United States until 1911. The name "Blattrollkrankheit" was first applied by APPEL in 1906. Since then the literature has become so voluminous that there has been much need of a monographic treatment of the subject.

This need has been met by the recent monograph of ESMARCH.¹⁴ After a brief review of the history, geographical distribution, and economic importance of leaf roll, he discusses the disease thoroughly under the headings (1) symptoms

¹² ASHTON, RUTH E., *Plants of Rocky Mountain National Park*. pp. iv+157. pls. 15. figs. 100. National Park Service, U.S. Dept. Interior. Washington, D.C. 1933.

¹³ CROWFOOT, GRACE M., and BALDENSPERGER, LOUISE, *From cedar to hyssop: A study in the folklore of plants in Palestine*. pp. viii+196. pls. 76. The Sheldon Press. London; Macmillan Company. New York. 1932. \$2.00.

¹⁴ ESMARCH, F., *Die Blattrollkrankheit der Kartoffel*. Julius Springer, Berlin. pp. 91. 1932.

and development of the disease, (2) histology, (3) physiology, (4) transmission, (5) influence of external factors, (6) internal disease factors, (7) etiology, and (8) resistance. The question of phloem necrosis is extensively discussed, the author having previously published on this subject as well as on the question of metabolism in plants affected with leaf roll. In the present monograph seven pages are devoted to the carbohydrate metabolism in diseased plants and two pages to protein metabolism, on which less is known. The monograph wisely deals only with publications occurring since 1911, literature previous to this time having been well summarized by APPEL (1911) and HIMMELBAUR (1912).—ALICE ALLEN BAILEY.

Statistical methods for research workers

The monograph by FISHER¹⁵ has appeared in a fourth edition. It is a very useful work for any biologist who must test the dependability of results by statistical procedures. The methods presented by FISHER for such work have the advantage that they are suitable for use with few samples. This will be appreciated, since it is often difficult to provide large numbers of samples.

The main additions to the work deal with covariance. The part played by covariance is emphasized mainly in chapter V, and a section on the analysis of covariance is added to chapter VIII. There are a few minor changes in chapter III on Distributions, and the appendix to this chapter has been entirely rewritten. As a whole the changes are too few to destroy the value of the previous edition of 1930 for those who have a copy of it.—C. A. SHULL.

Flora of South Dakota

A decided want has been met by OVER¹⁶ in compiling a check list of the vascular plants of South Dakota. The state presents a great variety of habitats, ranging from semidesert to mesophytic forests. Most unique of all are the Black Hills with their outliers from the Rocky Mountains and from the northern conifer forests. Although the present list gives little except the names of the species and the locations within the state where they are usually found, it will serve as a useful guide to future students. The illustrations are mostly half-tones from good photographs; but they are arranged in a rather disorderly fashion, without consecutive numbering and with curiously varied legends, so that their usefulness is much lessened. The terminology of RYDBERG's manual has been followed.—G. D. FULLER.

¹⁵ FISHER, R. A., *Statistical methods for research workers*. 8vo. pp. xvi+307. Oliver and Boyd. Edinburgh & London. 1932.

¹⁶ OVER, WM. H., *Flora of South Dakota*. pp. 161. figs. 75. University of South Dakota. Vermillion, S.D. \$1.00.

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